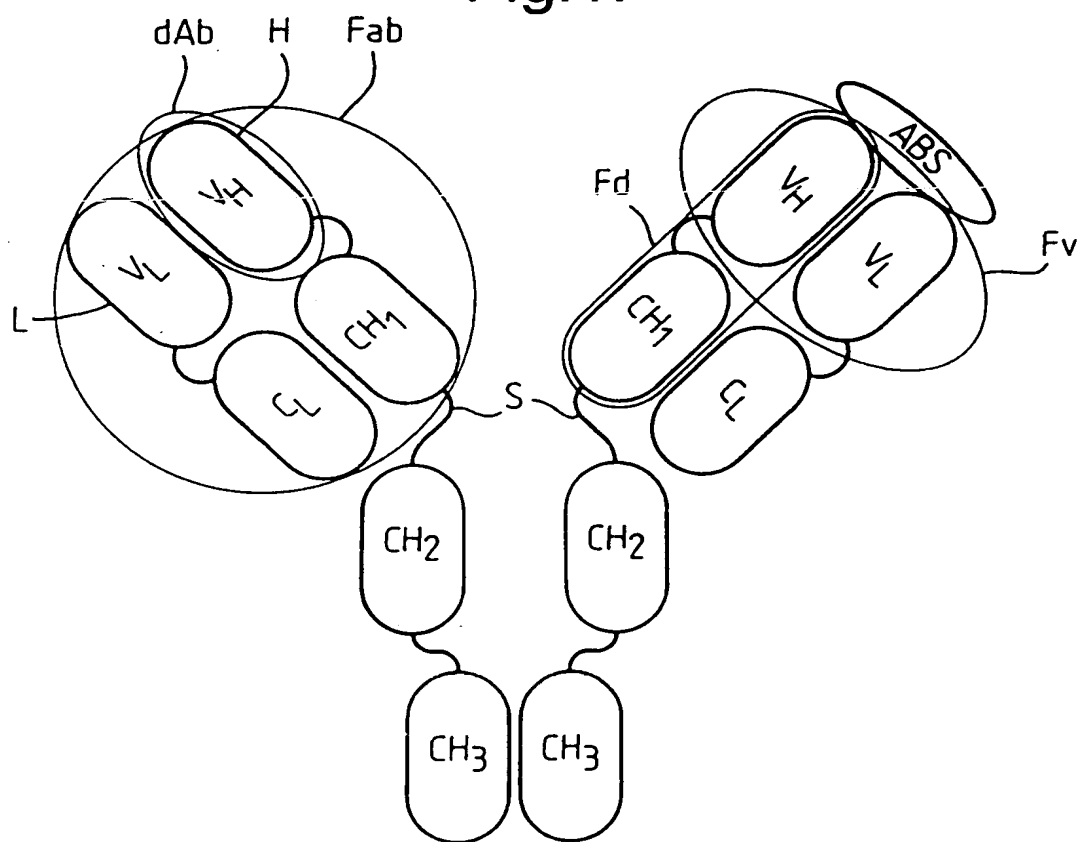
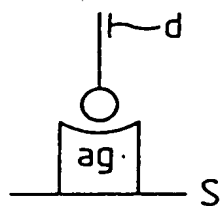


Fig.1.



$\rho$



$\text{O} \mid \text{P}$

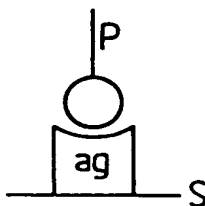
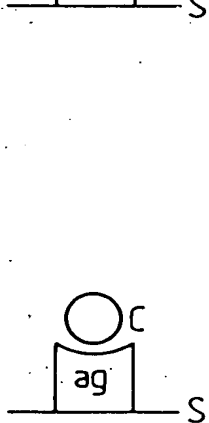
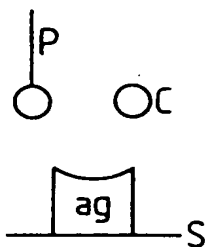
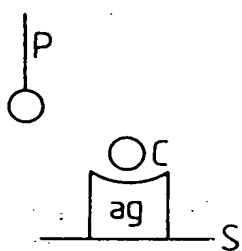
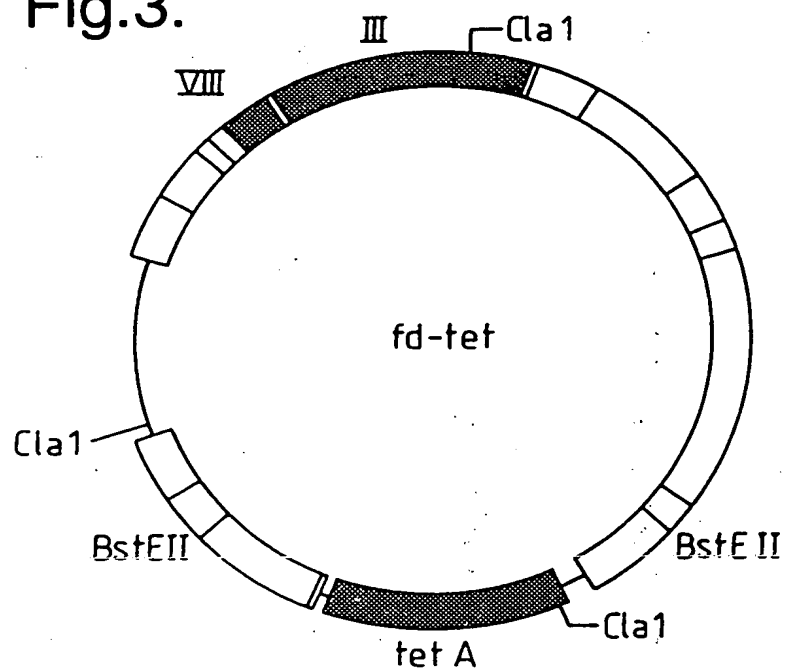


Fig.3.



fd - tet

~

cleave with BstEII

~

fill in with Klenow

~

re-ligate

↓

FDTδBst

~

in vitro mutagenesis (oligo 1)

↓

FDTPs/Bs

~

in vitro mutagenesis (oligo 2)

↓

FDTPs/Xh

60021 229900

(1653)  
 Oligo 1 ACA ACT TTC AAC AGT TGA GGA GAC GGT GAC CGT AAG CTT CTG CAG TTG GAC CTG AGC  
 GGA GTG AGA ATA (1620)  
 (1653)  
 Oligo 2 ACA ACT TTC AAC AGT TTC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG  
 (1704)  
 Oligo 3 GTC GTC TTT CCA GAC GTT AGT

Fig.4 (i).

GENE III  
 GENE III  
 SIGNAL  
 CLEAVAGE SITE  
 (1624)  
 A TCT CAC TCC GCT  
 Q V Q L Q V T V S S  
 B TCT CAC TCC GCT CAG GTC CAA CTG CAG AAG CTT ACC GTC ACC GTC TCC TCA ACT GTT GAA AGT  
 PstI BstEII  
 Q V Q L Q L E I K R  
 C TCT CAC TCC GCT CAG GTC CAA CTG CAG GAG CTC GAG ATC AAA CGG GAA ACT GTT GAA AGT  
 PstI XhoI

Fig.4 (ii).

Fig.5.

rbs M K Y L L P T A A  
 GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC  
 10 20 30 40 50 60  
 SphI  
 PelB leader  
 A G L L L L A A O P A M A Q V Q L Q E S  
 GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAGGAGTCA  
 70 80 90 100 110 120  
 PstI  
 G P G L V A P S Q S L S I T C T V S G F  
 GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC  
 130 140 150 160 170 180  
 S L T G Y G V N W V R Q P P G K G L E W  
 TCATTAACCGGCTATGGTGTAAACTGGGTTCCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG  
 190 200 210 220 230 240  
 VHD1.3  
 L G M I W G D G N T D Y N S A L K S R L  
 CTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTG  
 250 260 270 280 290 300  
 S I S K D N S K S Q V F L K M N S L H T  
 AGCATCAGCAAGGACAACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT  
 310 320 330 340 350 360  
 D D T A R Y Y C A R E R D Y R L D Y W G  
 GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC  
 370 380 390 400 410 420  
 Linker Peptide  
 Q G T T V T V S S G G G G S G G G G S G  
 CAAGGCACCAACGGTCAACCGTCTCCTCAggtggaggcggttcaggcgaggtggctctggc  
 430 440 450 460 470 480  
 BstEII  
 G G G S D I E L T Q S P A S L S A S V G  
 ggtggcgatcgGACATCGAGCTCACTCAGTCTCCAGCCTCCCTTTCTGCGTCTGTGGGA  
 490 500 510 520 530 540  
 SacI



Fig.6.

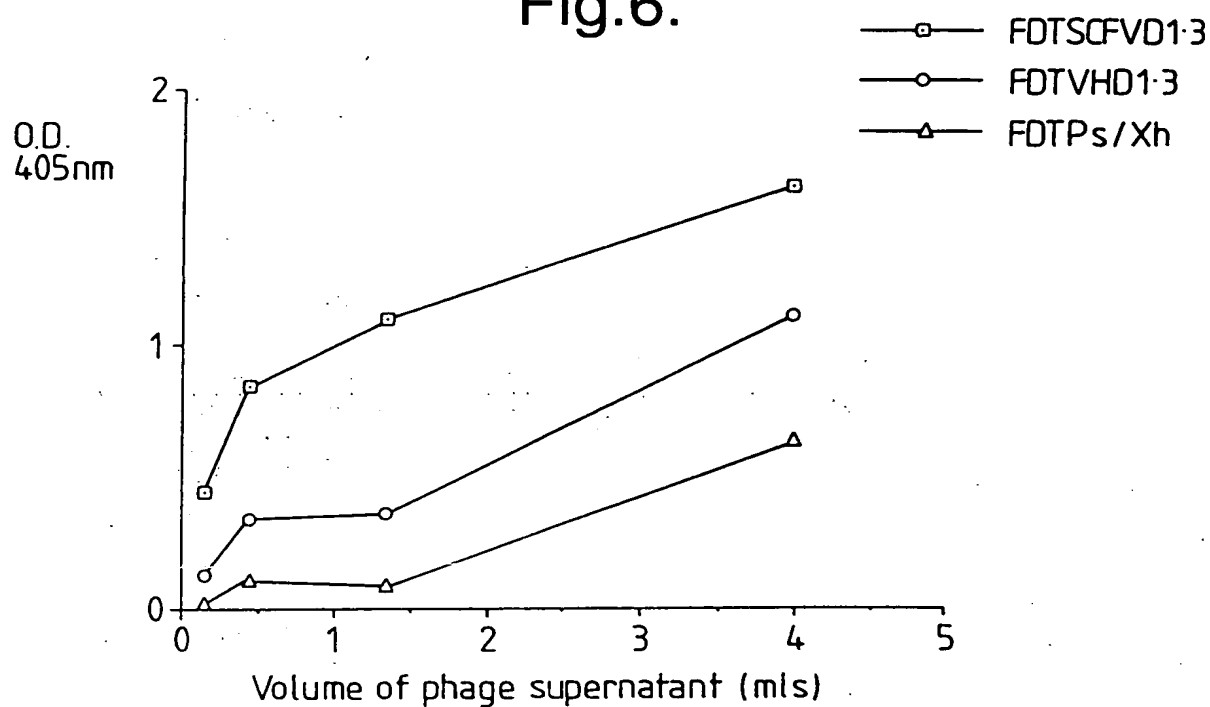


Fig.7.

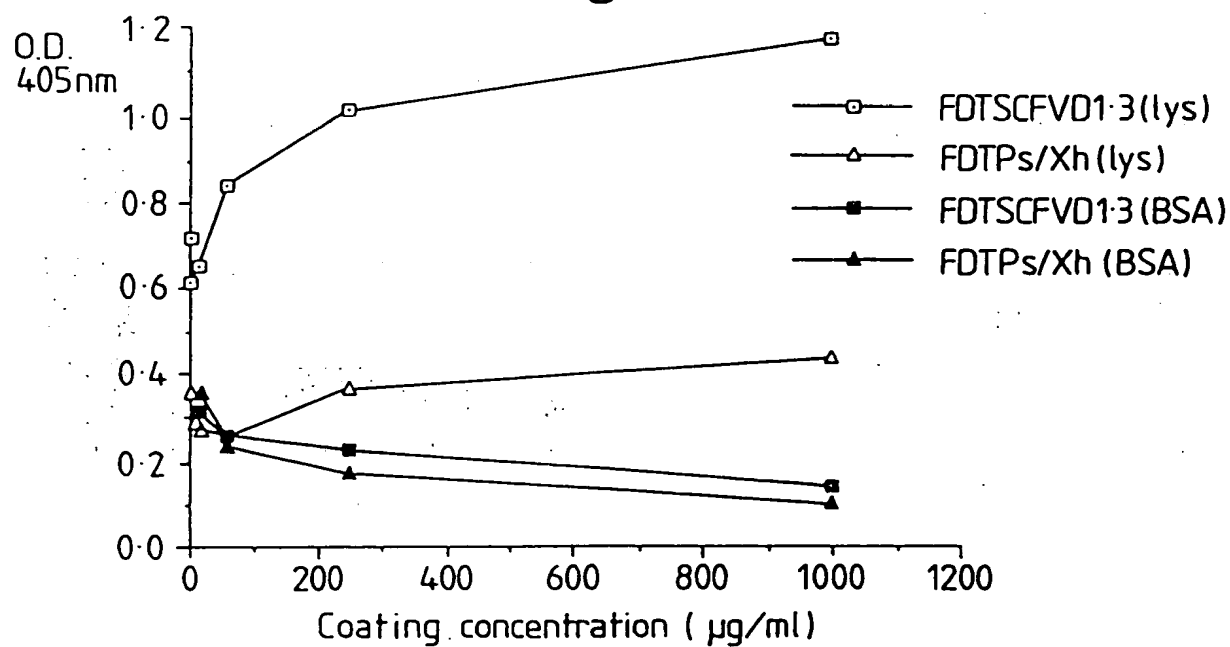


Fig.8.

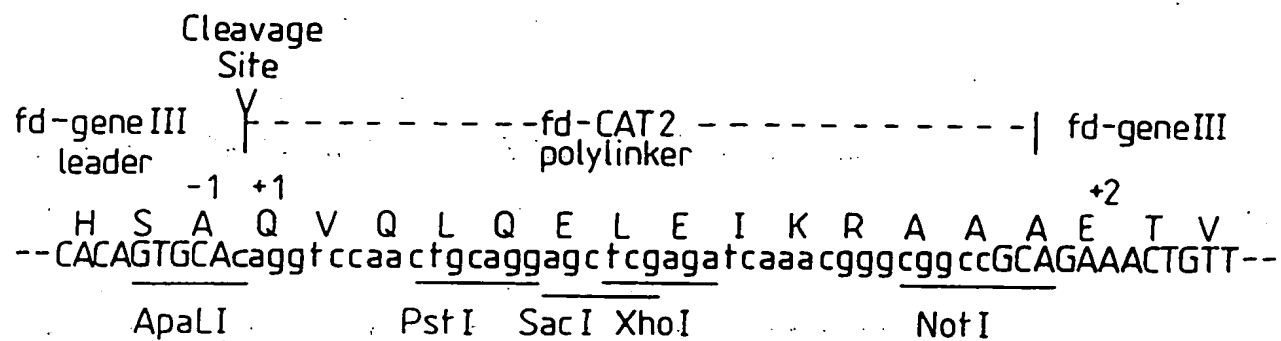


Fig.9.

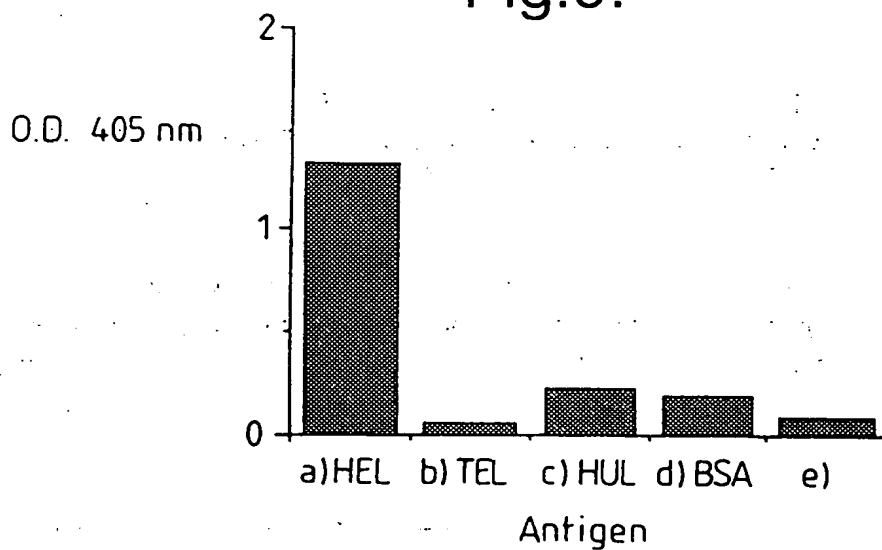




Fig.10.

GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC  
 10 20 30 40 50 60

A G L L L L A A Q P A M A Q V Q L Q E S  
 GCTGGATTGTTATTACTGCTGCCCAACCAGCGATGGCCCAAGTGCAGCTGCAGGAGTCA  
 70 80 90 100 110 120

G P G L V A P S Q S L S I T C T V S G F  
 GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC  
 130 140 150 160 170 180

S L T G Y G V N W V R Q P P G K G L E W  
 TCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTCGAGTGG  
 190 200 210 220 230 240

L G M I W G D G N T D Y N S A L K S R L  
 CTGGGAATGATTTGGGGTGATCGAAACACAGACTATAATTACAGCTCTCAAATCCAGACTG  
 250 260 270 280 290 300

S I S K D N S K S Q V F L K M N S L H T  
 AGCATCAGCAAGGACAACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT  
 310 320 330 340 350 360

D D T A R Y Y C A R E R D Y R L D Y W G  
 GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC  
 370 380 390 400 410 420

Q G T T V T V S S A S T K G P S V F P L  
 CAAGGCACCAAGGTCAACGCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG  
 430 440 450 460 470 480

A P S S K S T S G G T A A L G C L V K D  
 GCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGGGCCCTGGGCTGCCTGGTCAAGGAC  
 490 500 510 520 530 540

Fig.10 (Cont 1).

Y F P E P V T V S W N S G A L T S G V H  
TACTTCCCCGAACCGGTGACGGTGTCTGTGGAATCAGGCGCCCTGACCAGCGGGGTGCAC  
550 560 570 580 590 600

T F P A V L Q S S G L Y S L S S V V T V  
ACCTTCCCCGGCTGTCTACAGTCTCTAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG  
610 620 630 640 650 660

P S S S L G T Q T Y I C N V N H K P S N  
CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC  
670 680 690 700 710 720

T K V D K K V E P K S S \* \*  
ACCAAGGTGACAAGAAAGTTGAGCCCAATCTTCAATAAACC CGGAGCTTGCATGCA  
730 740 750 760 770 780

M K Y L L P T A A A G L  
AATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCCTACGGCAGCCGCTGGAT  
790 800 810 820 830 840

L L L A A Q P A M A D I E L T Q S P A S  
TGTTATTACTGCTGCCCCAACCAGCGATGGCCGACATCGAGCTACCCAGTCTCCAGCCT  
850 860 870 880 890 900

L S A S V G E T V T I T C R A S G N I H  
CCCTTTCTGGTCTGTGGGAGAACTGTACCATCACATGTGAGCAAGTGGGAATATT  
910 920 930 940 950 960

N Y L A W Y Q Q K Q G K S P Q L L V Y Y  
ACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATT  
970 980 990 1000 1010 1020

# Fig.10 (Cont 2).

T T T L A D G V P S R F S G S G S G T Q  
ATACAACAACCTTAGCAGATGGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGAACAC  
1030 1040 1050 1060 1070 1080

Y S L K I N S L Q P E D F G S Y Y C Q H  
AATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACIGTCAAC  
1090 1100 1110 1120 1130 1140

F W S T P R T F G G G T K L E I K R T V  
ATTTTTGGGAGTACTCCTCGGACGTTGGTGGAGGCACCAAGCTCGAGATCAAACGGACTG  
1150 1160 1170 1180 1190 1200

A A P S V F I F P P S D E Q L K S G T A  
TGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGCTG  
1210 1220 1230 1240 1250 1260

S V V C L L N N F Y P R E A K V Q W K V  
CCTCTGTGTGTGCTGTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGGAAGG  
1270 1280 1290 1300 1310 1320

D N A L Q S G N S Q E S V T E Q D S K D  
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTTCACAGAGCAGGACAGCAAGG  
1330 1340 1350 1360 1370 1380

S T Y S L S S T L T L S K A D Y E K H K  
ACAGCACCTACAGCCTCAGCAGCACCCCTGAAGCTGAGCAAAGCAGACTACGAGAAACACA  
1390 1400 1410 1420 1430 1440

V Y A C E V T H Q G L S S P V T K S F N  
AAGTCTACGCCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCA  
1450 1460 1470 1480 1490 1500

R G E S \* \*  
ACCGCGGAGAGTCATAGTAAGAATTC  
1510 1520

0049567 11300  
360211 22556760

Fig.10 (Cont 3).

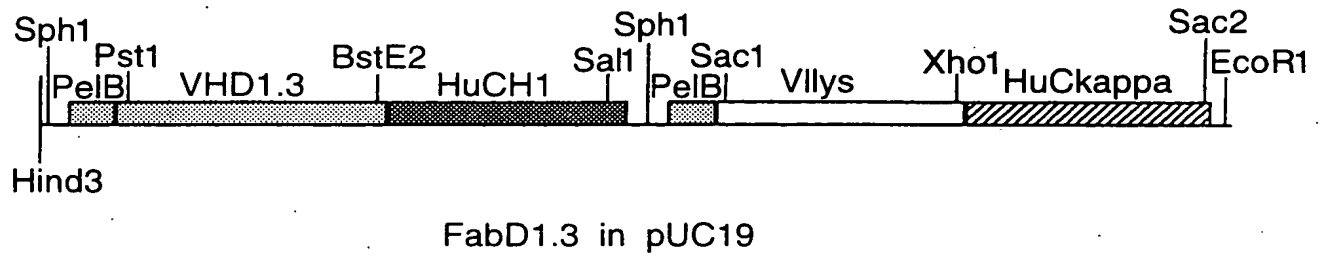
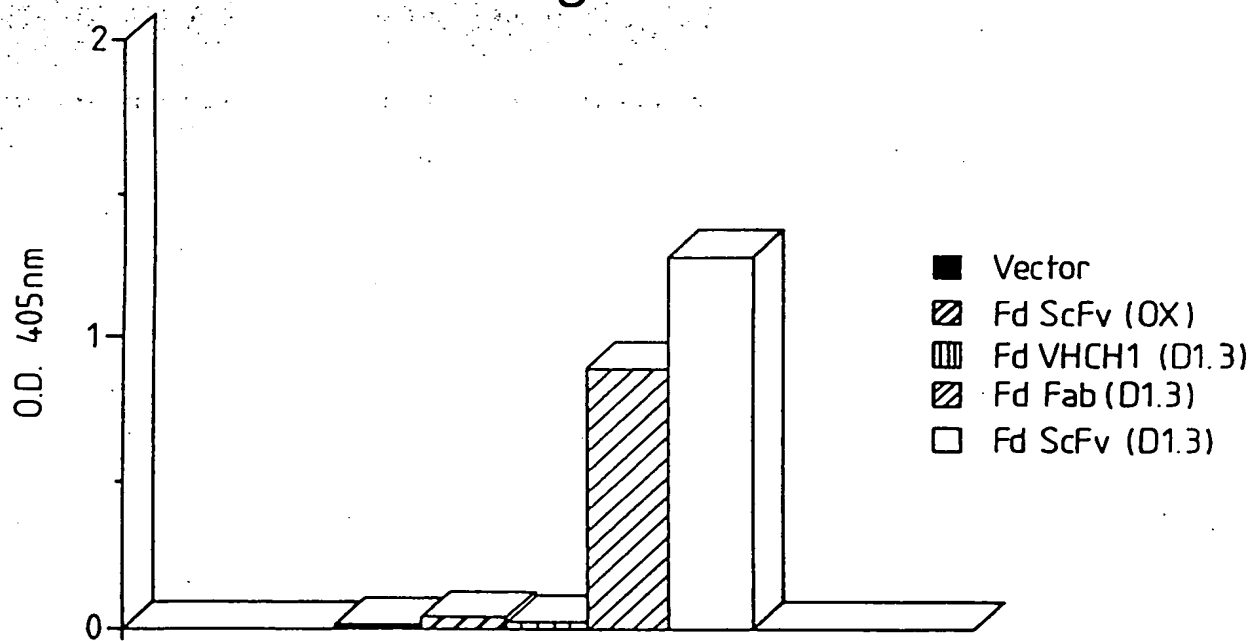


Fig.11.






Fig.13.

Q V Q L Q E S G G G L V Q P G G  
CAG GTG CAG CTG CAG GAG TCA GGA GGA GGC TTG GTA CAG CCT GGG GGT  
PstI  
S L R L S C A T S G F T F S N Y  
TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT AAT TAC  
Y M G W V R Q P P G K A L E W L  
TAC ATG GGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG  
G S V R N K V N G Y T T E Y S A  
GGT TCT GTT AGA AAC AAA GTT AAT GGT TAC ACA ACA GAG TAC AGT GCA  
S V K G R F T I S R D N F Q S I  
TCT GTG AAG GGG CGG TTC ACC ATC TCC AGA GAT AAT TTC CAA AGC ATC  
L Y L Q I N T L R T E D S A T Y  
CTC TAT CTT CAA ATA AAC ACC CTG AGA ACT GAG GAC AGT GCC ACT TAT  
Y C A R G Y D Y G A W F A Y W G  
TAC TGT GCA AGA GGC TAT GAT TAC GGG GCC TGG TTT GCT TAC TGG GGC  
Q G T L V T v s s g g g g s g g g g s  
CAA GGG ACC CTG GTC ACC gtc tcc tca ggtggaggcggttcaggcgagggtggctct  
BstEII  
g g g g s d i E L T Q T P L S L P V  
ggcggtggcggtcgac atc GAG CTC ACC CAA ACT CCA CTC TCC CTG CCT GTC  
SacI  
S L G D Q A S I S C R S S Q S I  
AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT  
V H S N G N T Y L E W Y L Q K P  
GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA  
PstI  
G Q S P K L L I Y K V S N R F S  
GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT  
G V P D R F S G S G S G T D F T  
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCG GGG ACA GAT TTC ACA  
L K I S R V E A E D L G V Y Y C  
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC  
F Q G S H V P Y T F G G G T K L  
TTT CAA GGT TCA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTC  
E I K R  
GAG ATC AAA CGG  
XhoI

02106673 41009

Fig.14.

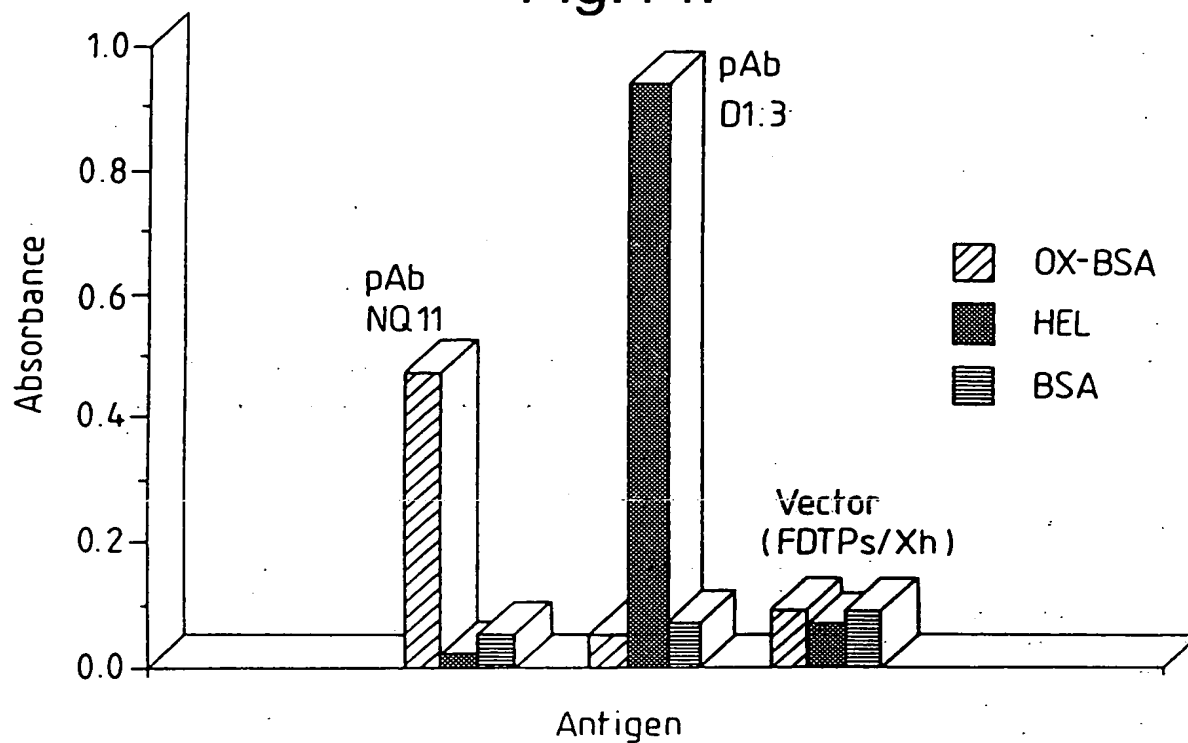


Fig.15.

5' END

TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG  
 ApaL1

3' END

K A A L G L K  
 AAA GCC GCT CTG GGG CTG AAA GCG GCC GCA GAA ACT GTT GAA AGT etc.  
 Not I

Fig.16 (i).

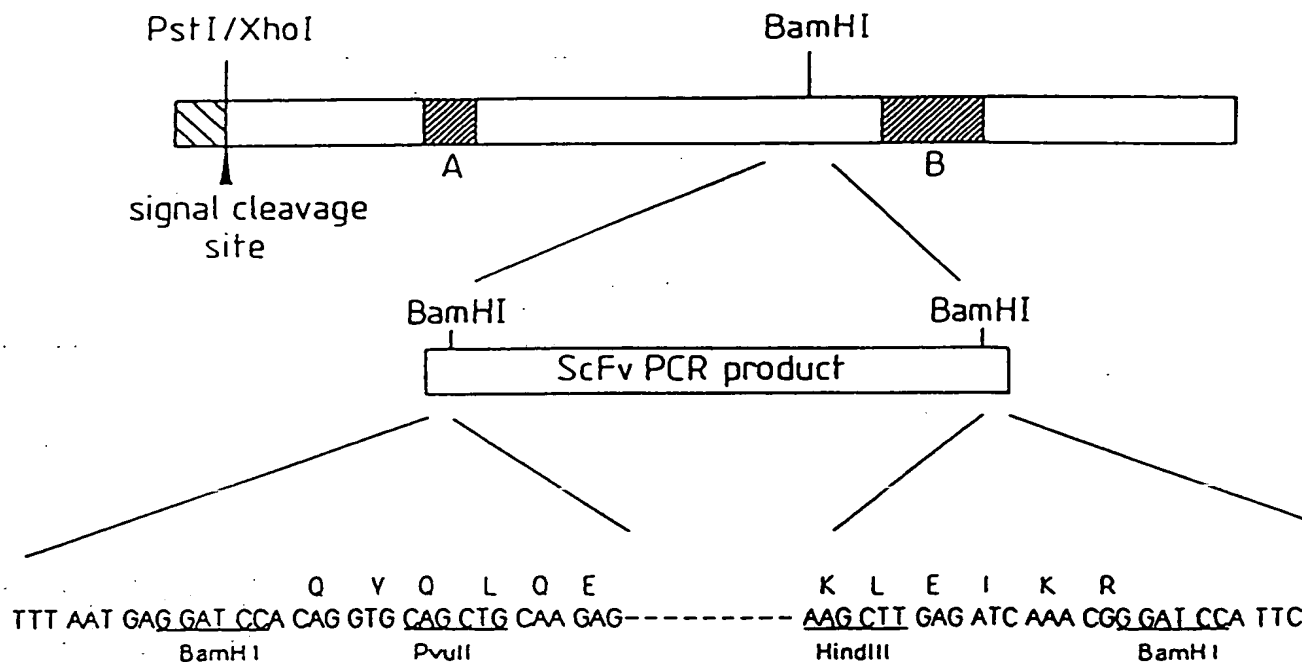


Fig.16 (ii).

A (1834) 5' GAG GGT GGT GGC TCT  
 " " "C " "  
 " " "C " "  
 " " "C " ACT 3'(1839)

B (2284) 5' - GGC GGC GGC TCT  
 - GGT GGT GGT "  
 - " GGC GGC "  
 GAG " " GGC "  
 " " " GGT "  
 " " " GGC "  
 - " " GGT "  
 - " " GGC " 3'(2379)

Reverse complement of mutagenic  
 oligo G3Bamlink

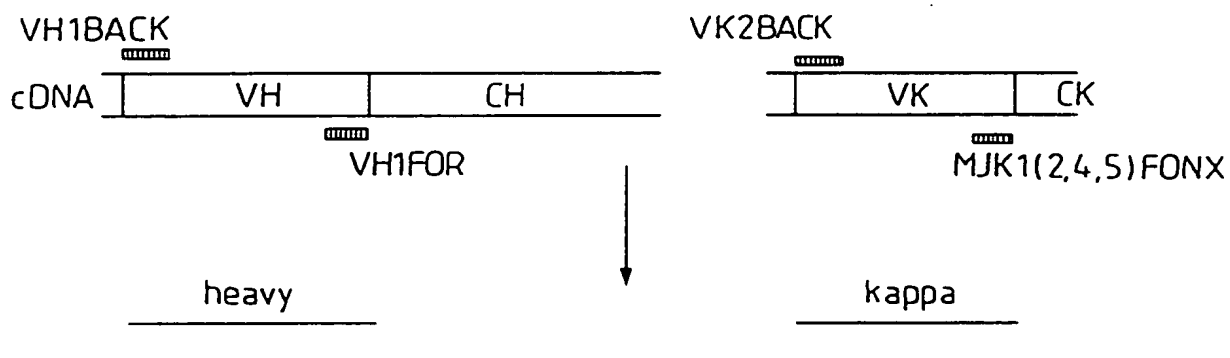
5' GAG GGT GGC GGA TCC

GAG GGT GGC GG 3'

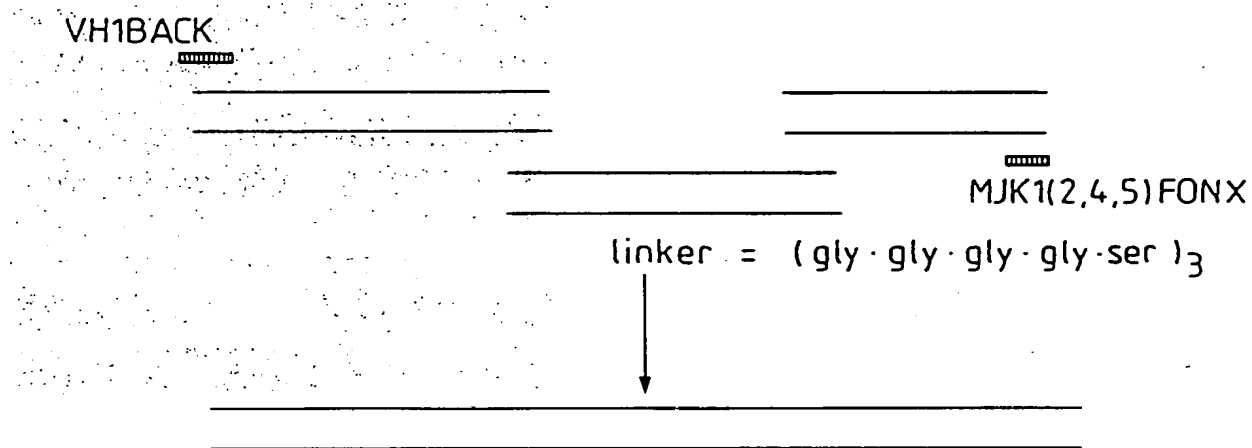


Fig.17.

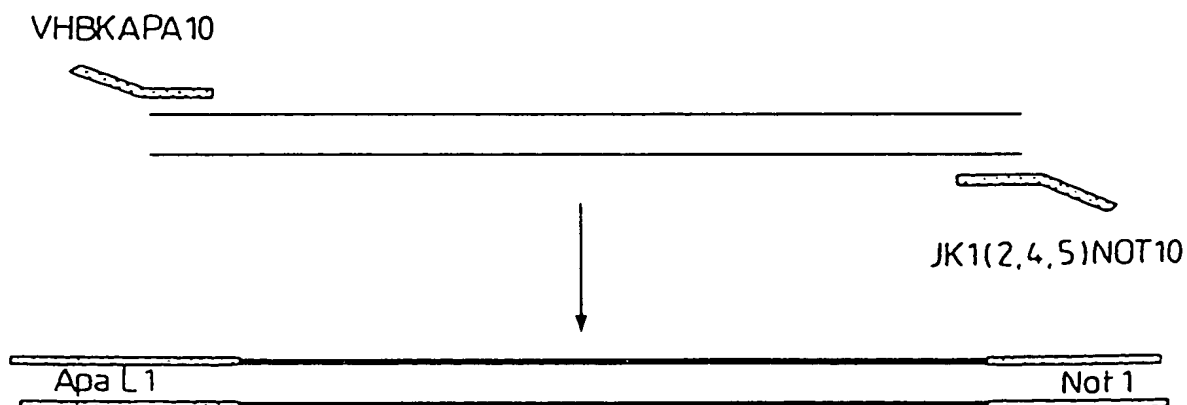
1) PRIMARY PCR



2) ASSEMBLY PCR



3) ADDING RESTRICTION SITES



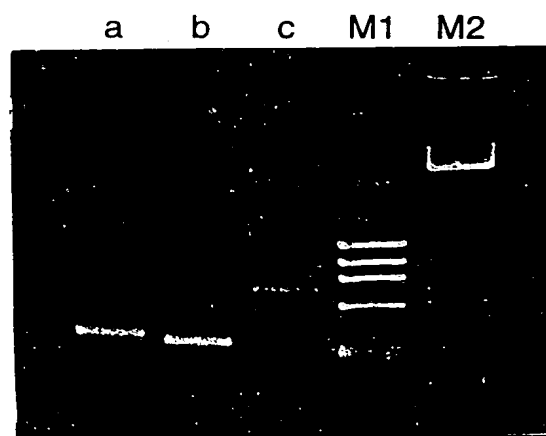
[illegible]

Fig.19.

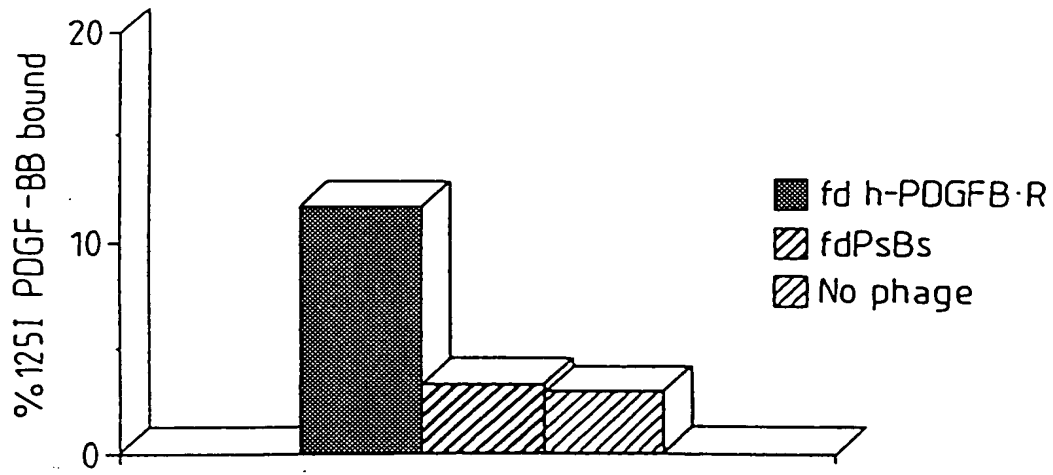
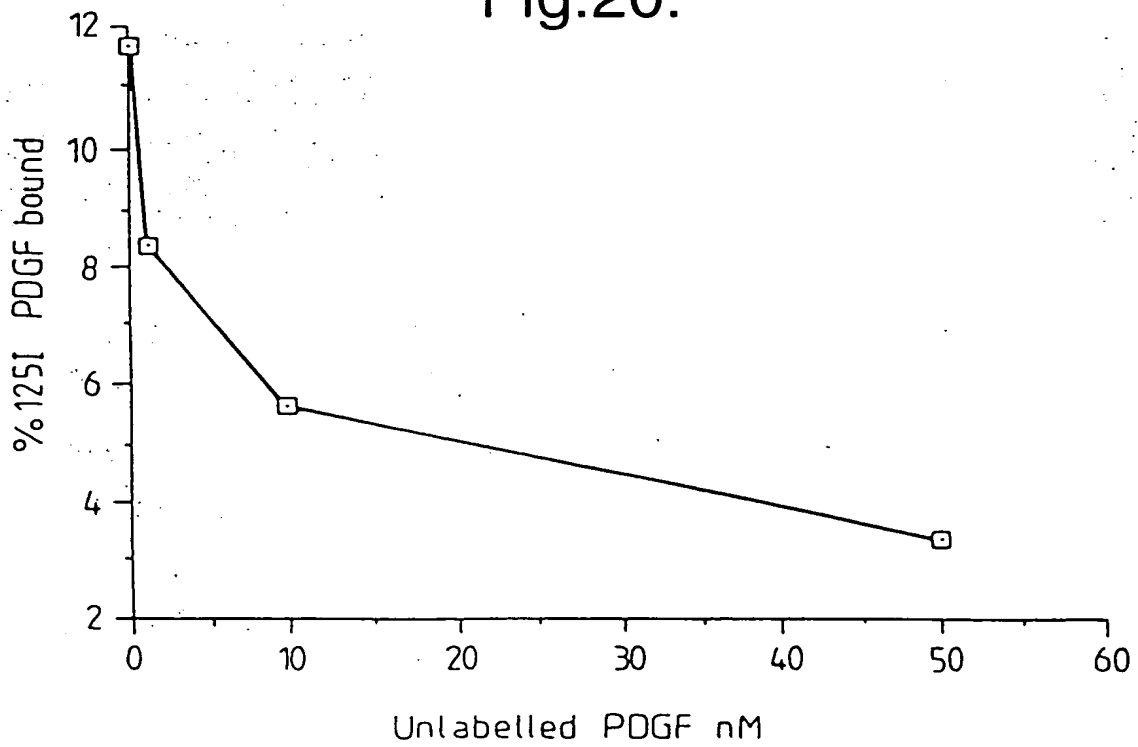
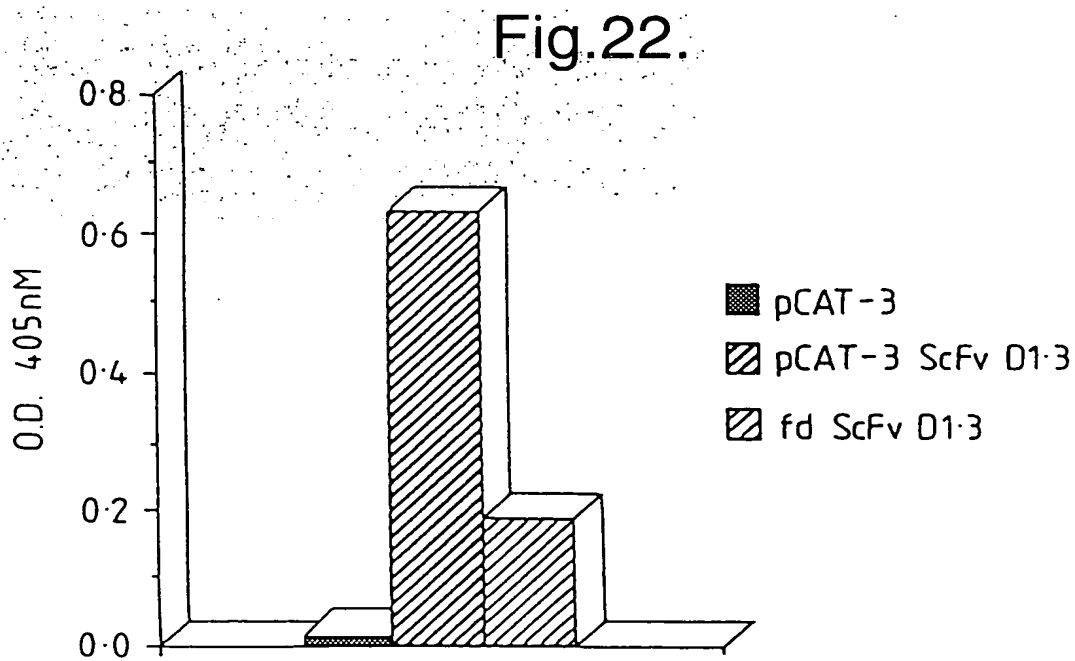
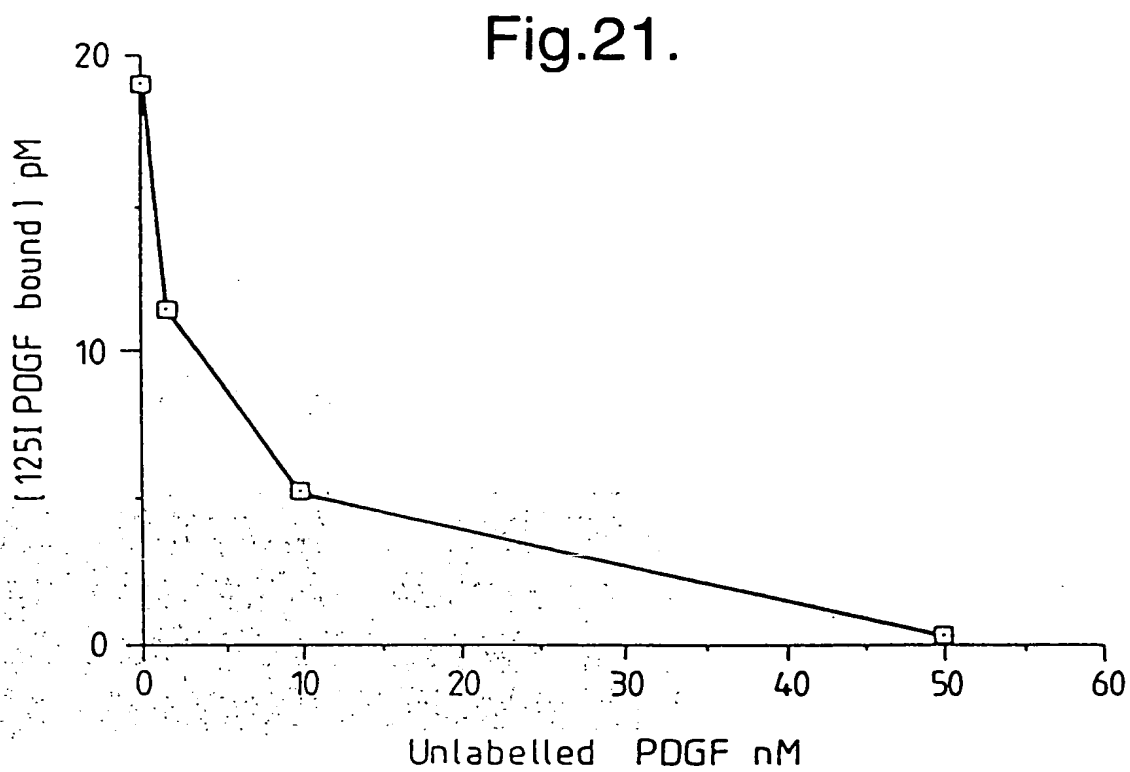


Fig.20.





0949537 112093  
060277 E2356T60

Fig.23(i )

d

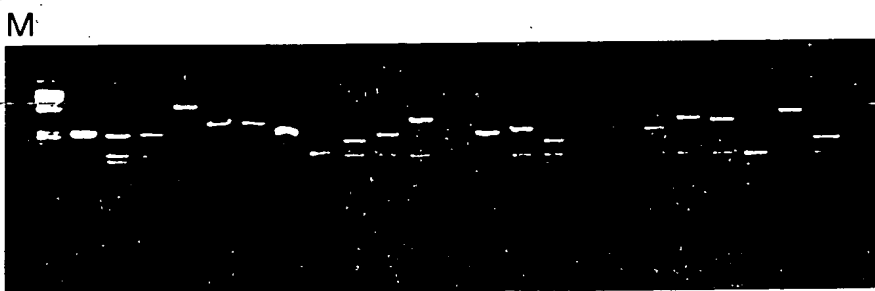


Fig.23(ii)

M

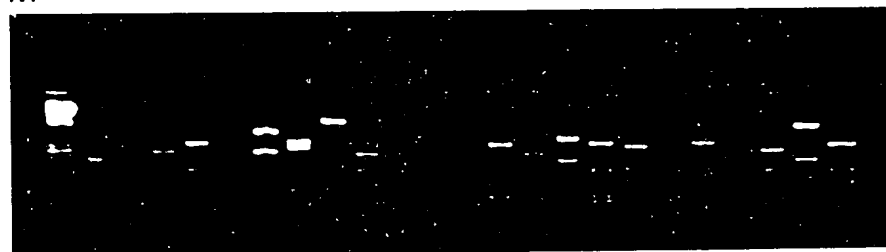


Fig.24.

VH sequences

from combinatorial library:

	CDR1		CDR2		CDR3	
A	QVQLQQSGAELARPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x4
B	QVQLQQSGAELAKPGASVVKMSCKASGYTFT	HLKQRPQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x9
C	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x3
D	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	RINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x3
E	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	2 V <sub>H</sub> oxJ
F	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
G	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
H	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPNDGTYNEXKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1

from hierarchical library VH-rep x Vκ-d:

I	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
J	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
K	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x3
L	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x2
M	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
N	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
O	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
P	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
Q	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
R	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x2
S	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	x6
T	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
U	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1
V	QVQLQQSGPELVKPGASVVKMSCKASGYTFT	WVKRPPQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	HQOQTIVTVSS	1

## Vx sequences

**from combinatorial library:**

20190327 09:36:00

Fig.25.

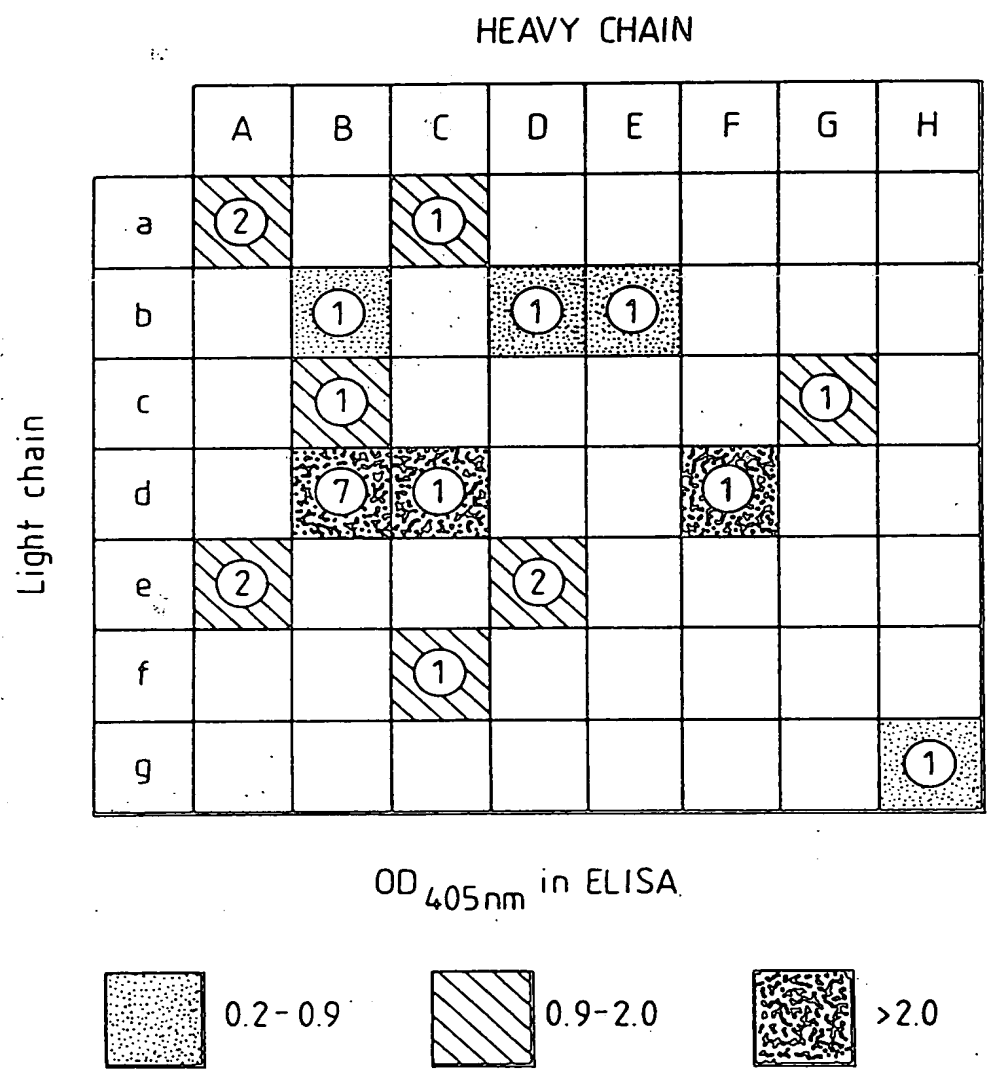




Fig.26(a).

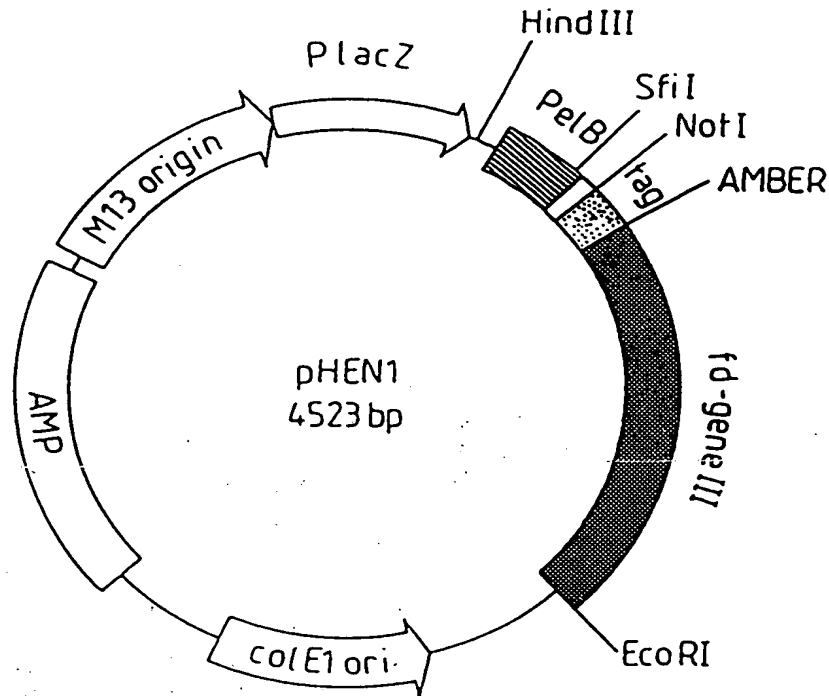


Fig.26(b).

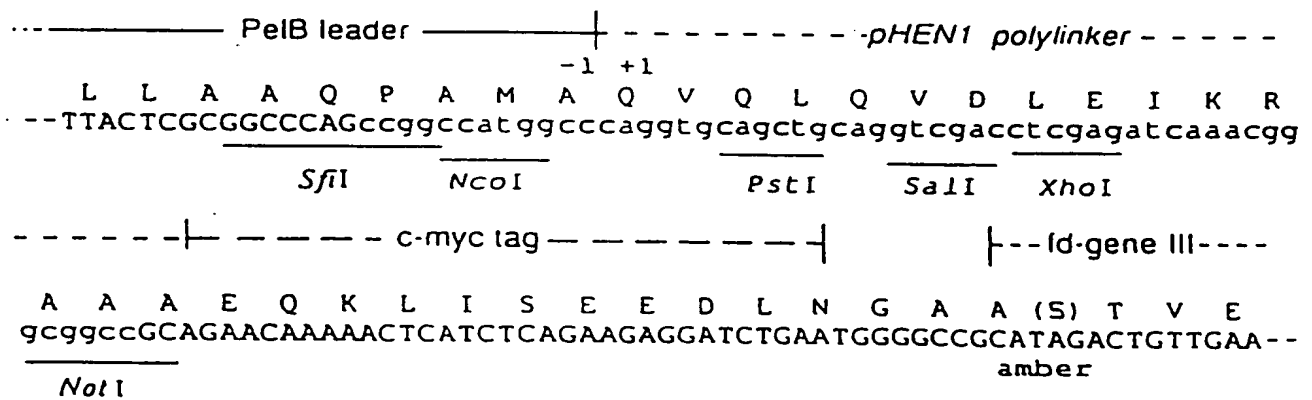
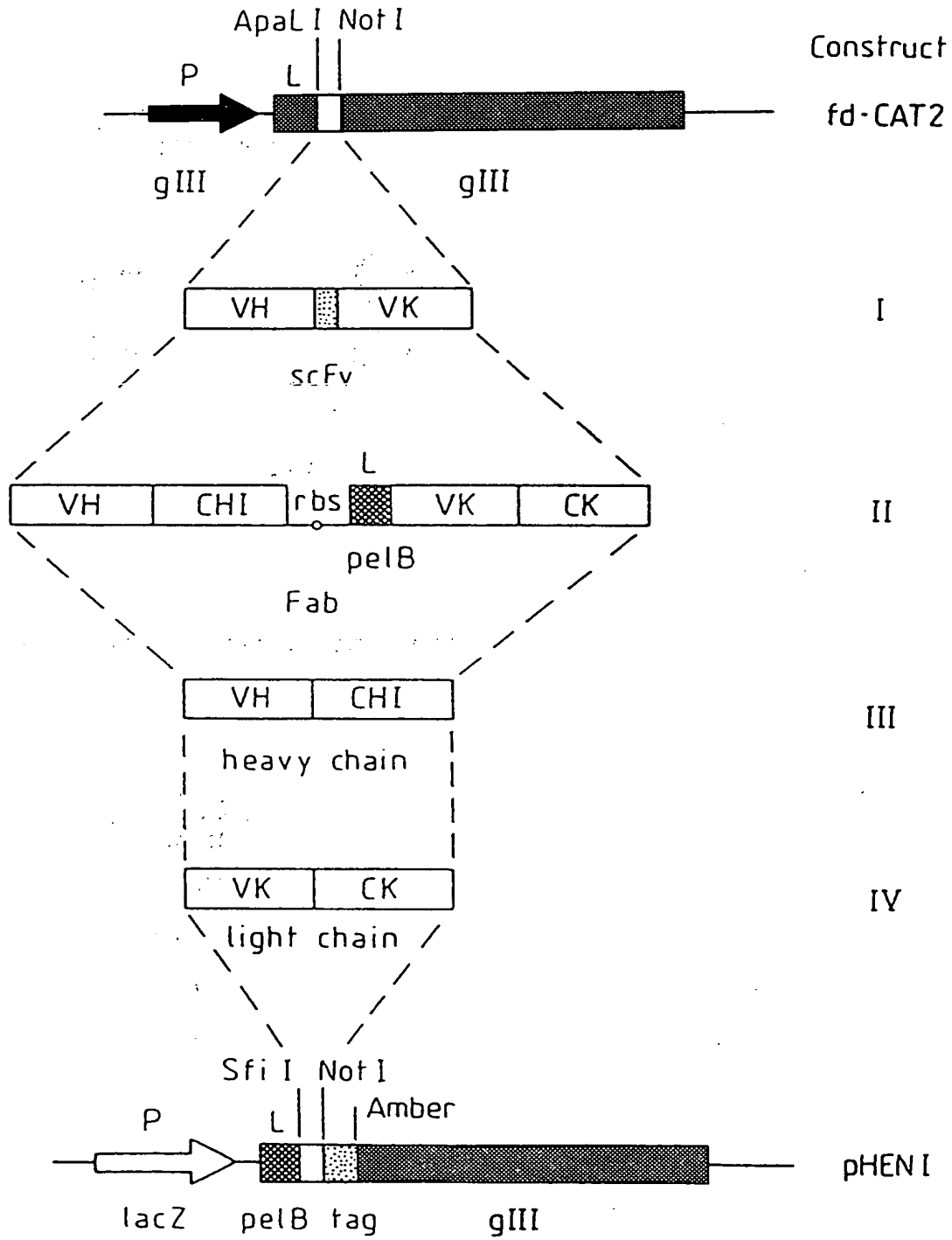


Fig.27.



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Fig.28.

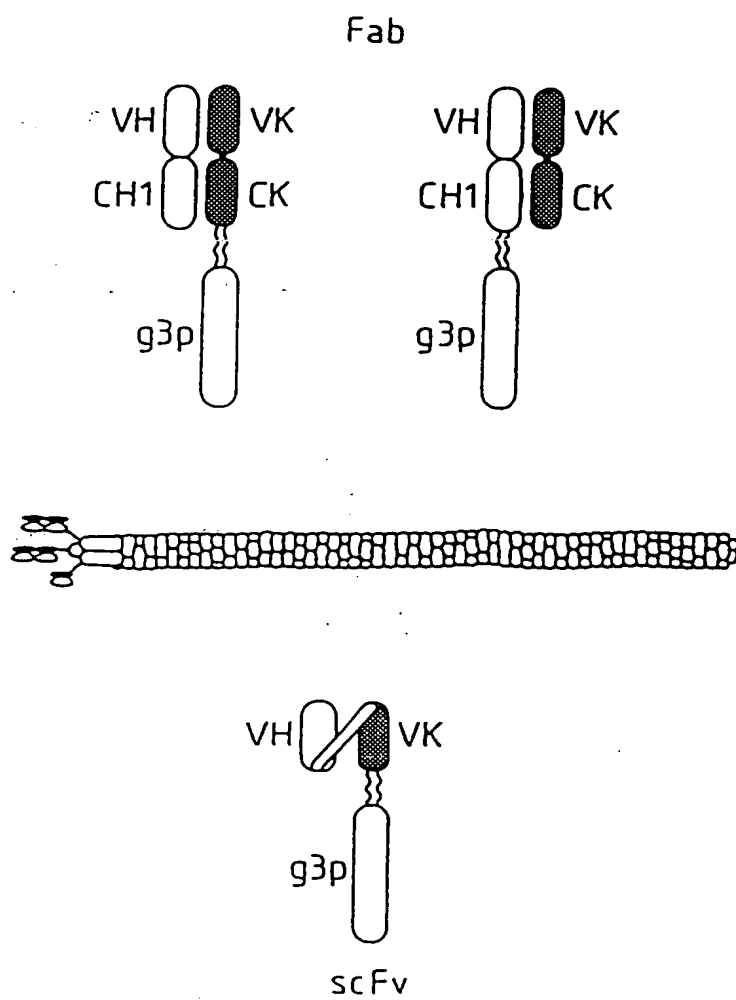


Fig.29.

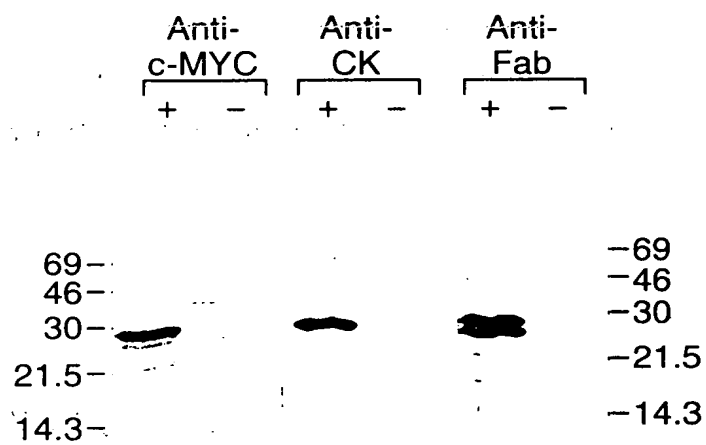


Fig.30.

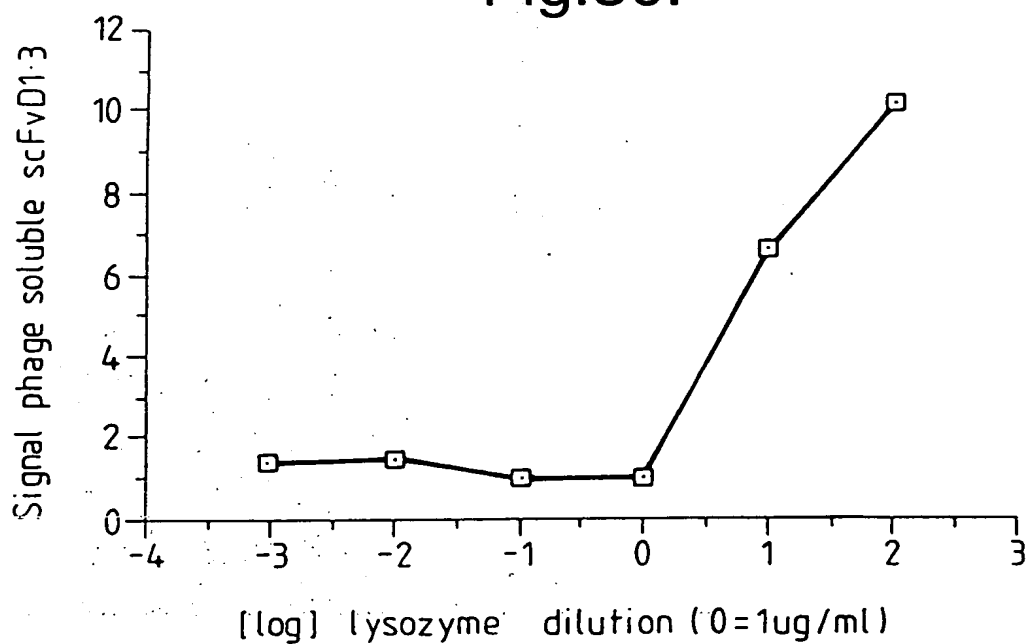


Fig.31.

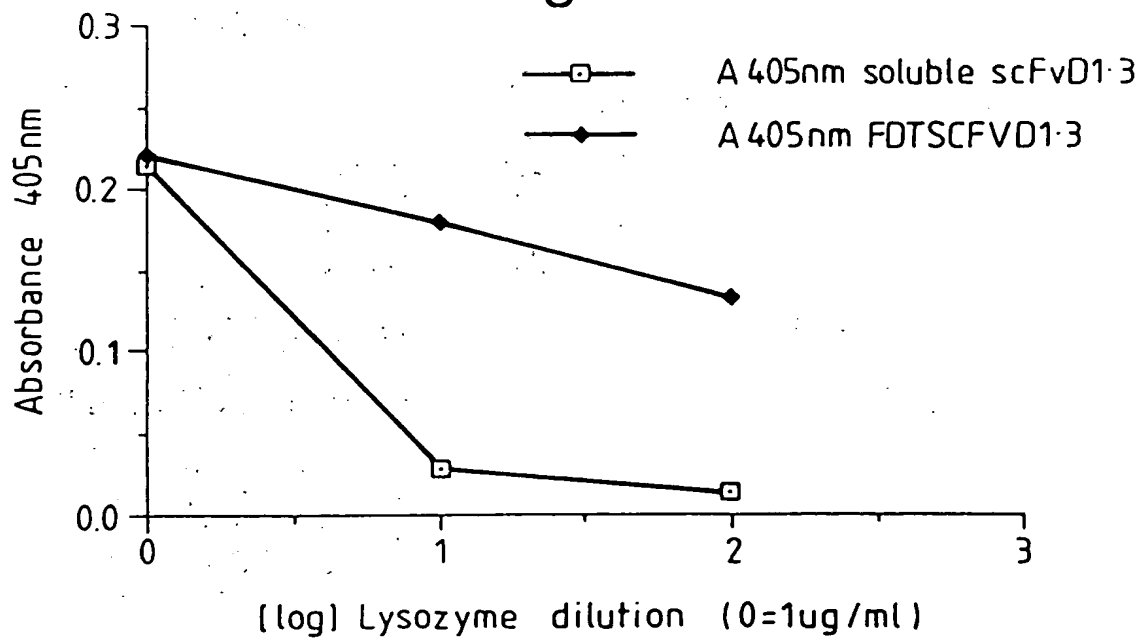


Fig.32.

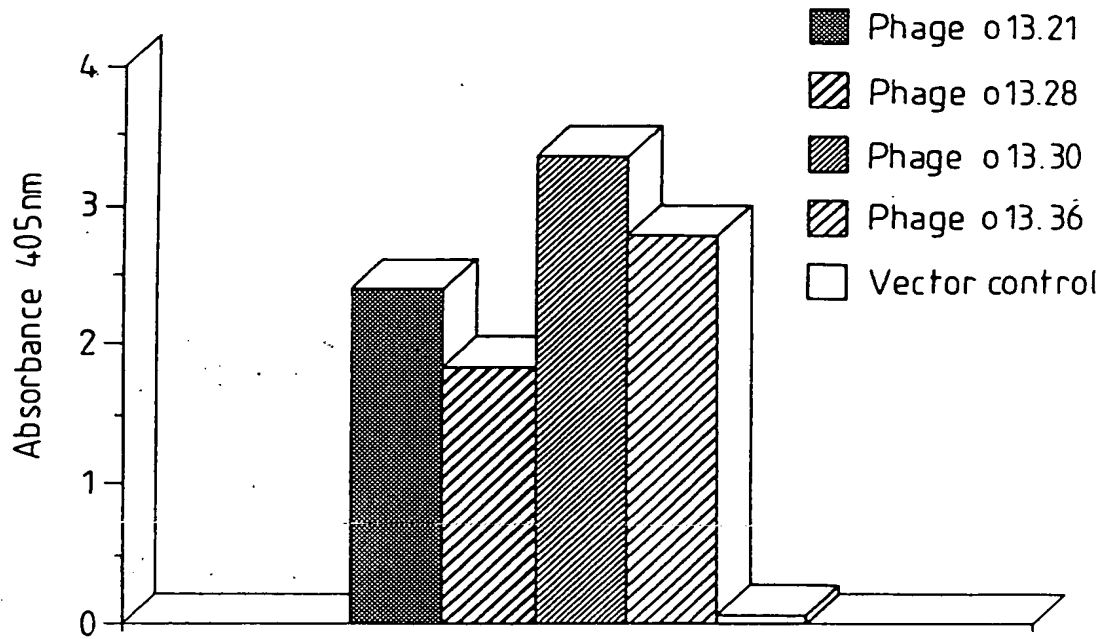


Fig.33.

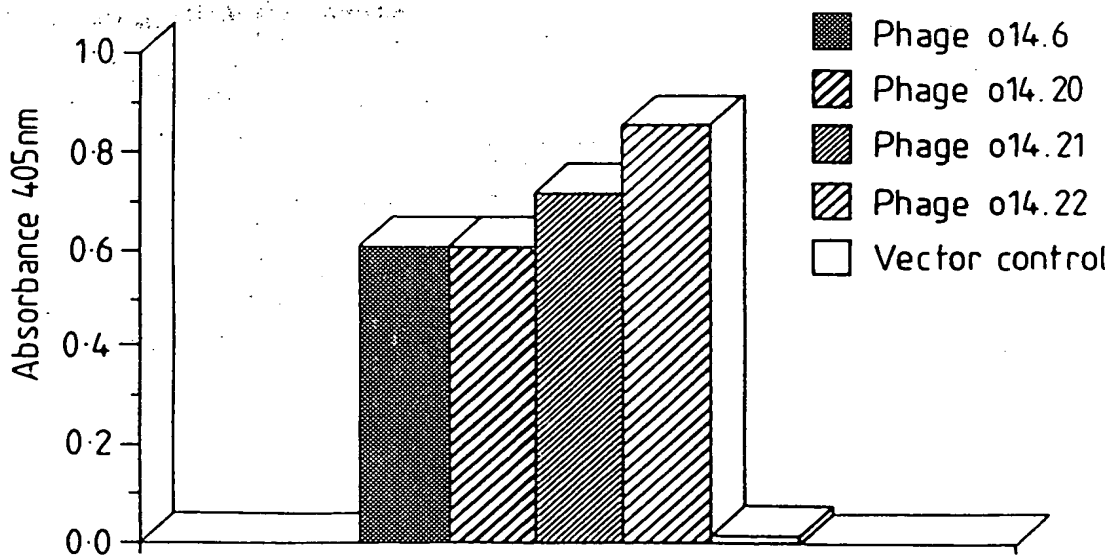


Fig.34.

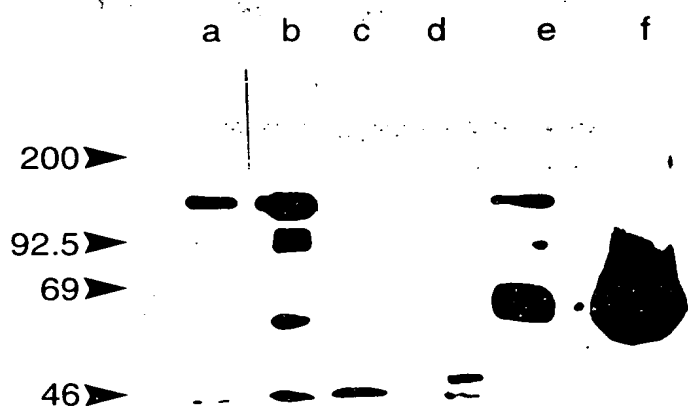


Fig.35A.

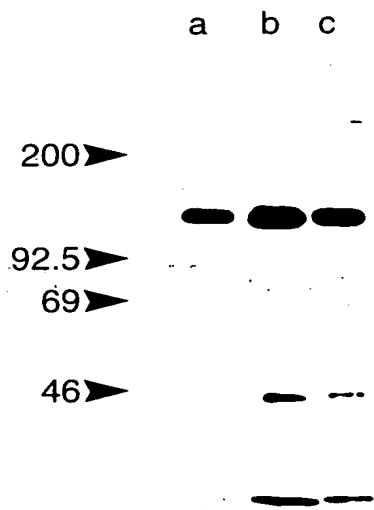


Fig.35B.

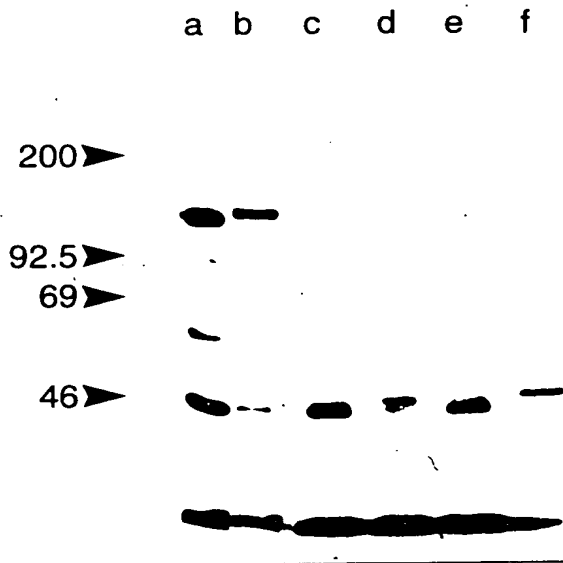


Fig.36.

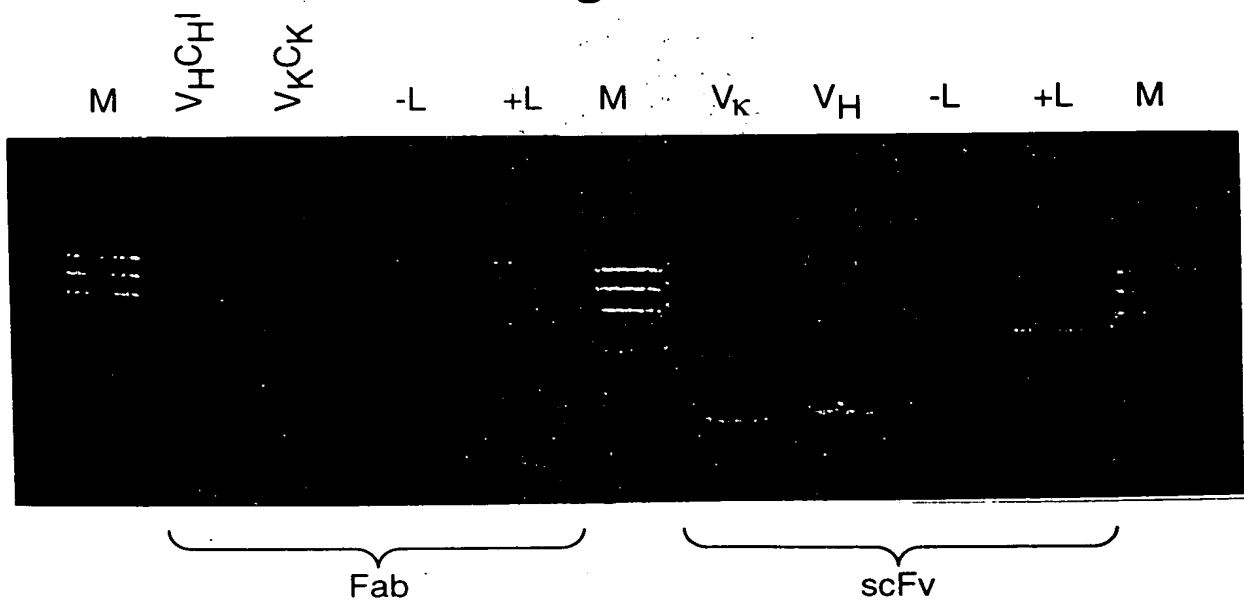




Fig.37.

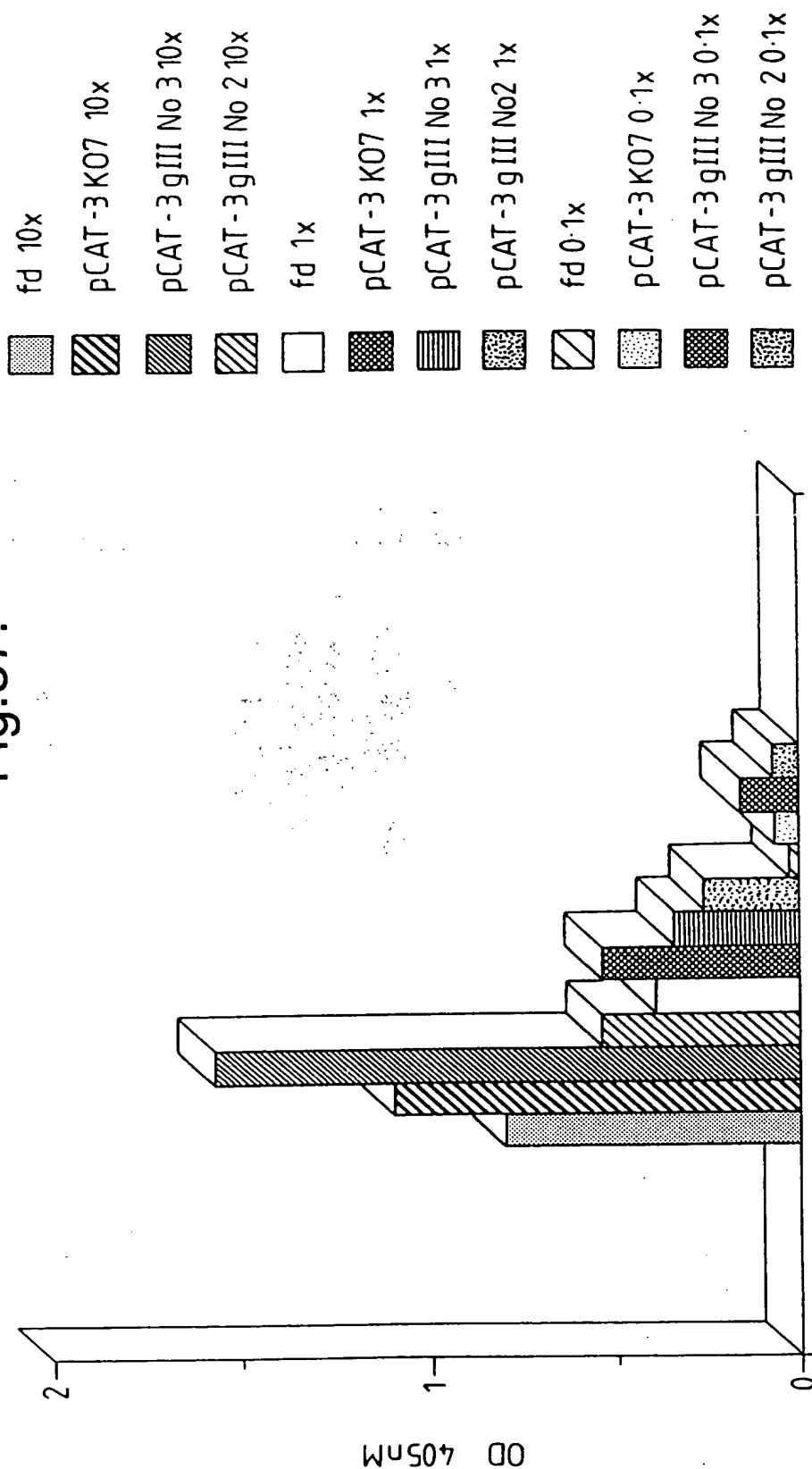


Fig.38A.



Fig.38B.

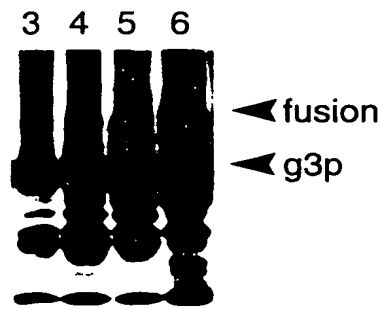


Fig.39.

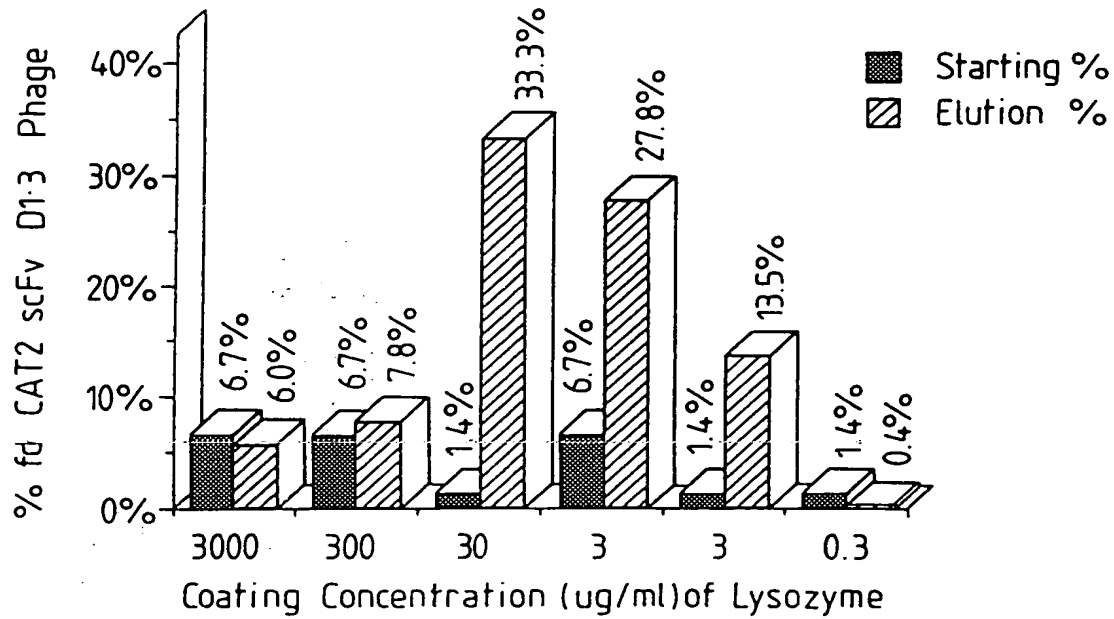
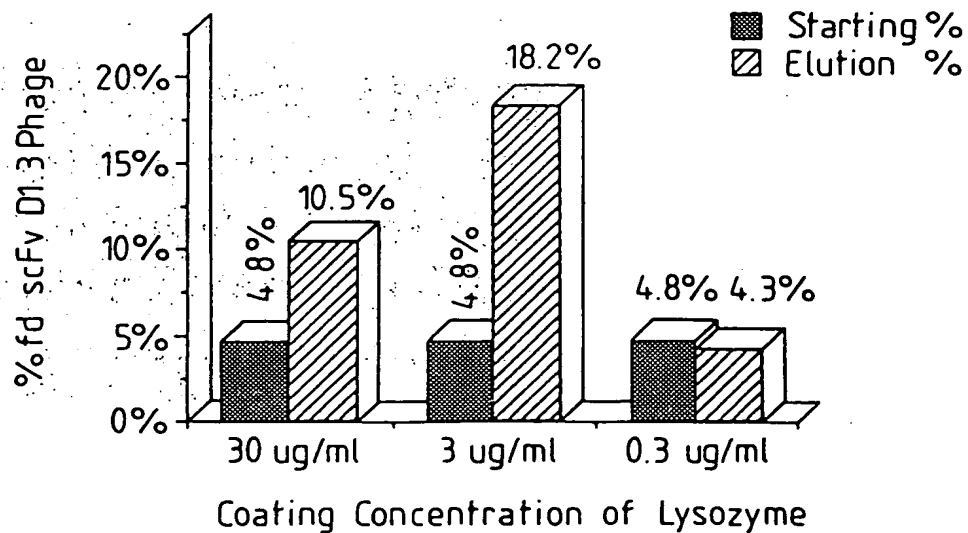


Fig.40.



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Fig.41.

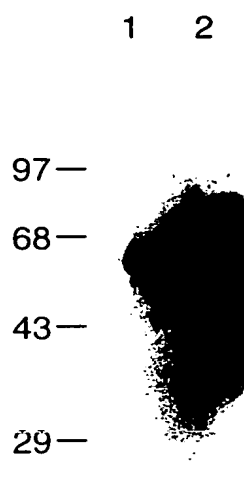


Fig.42.

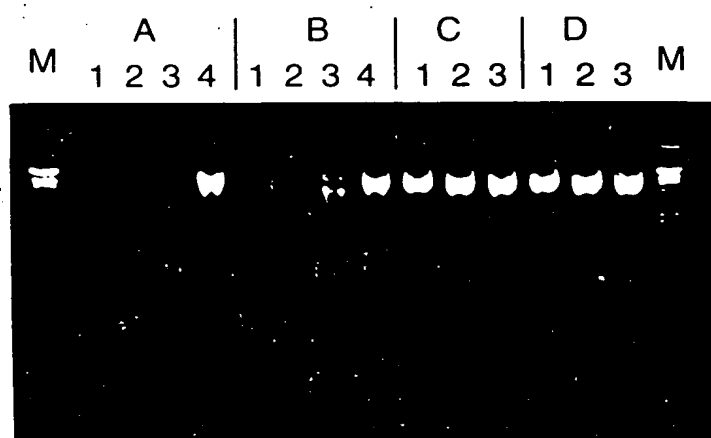
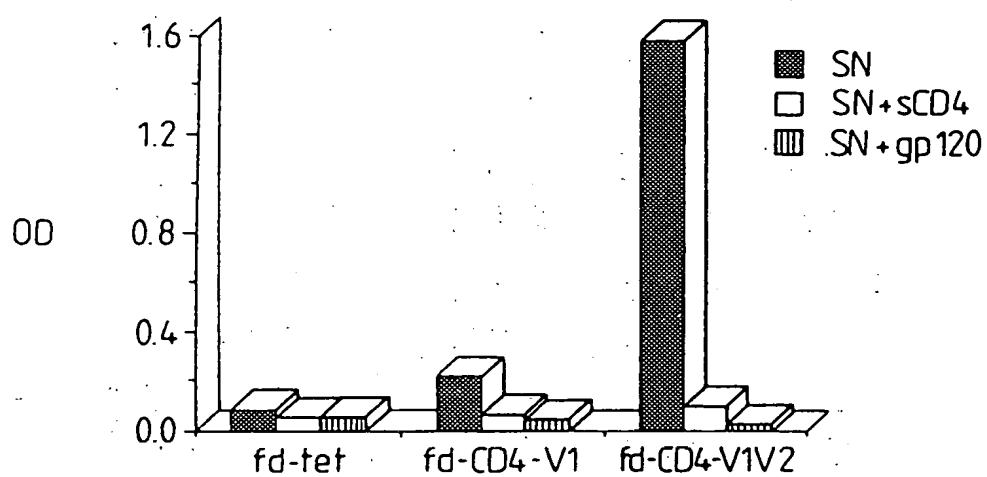


Fig.43.





640 650 660 670 680 690 700 C G 710 720  
GGAGACAAGGCTGCCCTCACCATCACAGGGGCACAGACTGAGGATGAGGCAATATTTCTGTGCTCTATGGTACAGCAACCATTTGGGTG  
CCTCTGTTCCGACGGAGTGTGTCTCCCGTGTCTGACTCCTACTCCGTTATATAAGACACGAGATACCATGTCTGTTGGTAACCCAC  
GlyAspLysAlaAlaLeuThrIleThrGlyAlaGlnThrGluAspGluAlaIleTyrPheCysAlaLeuTrpTyrnberAsnHisTrpVal

730 740 750 760 770  
TTCGGTGAGGAACCAAACTGACTGTCTCTCGAGATCAAACGGGGCGCCGC  
AAGCCACCTCCTTGTTGACTGACAGGAGCTCTAGTTTGCCCGCCGGCG  
PheGlyGlyGlyThrLysLeuThrValLeuGluIleLysArgAlaAla

Fig.45.

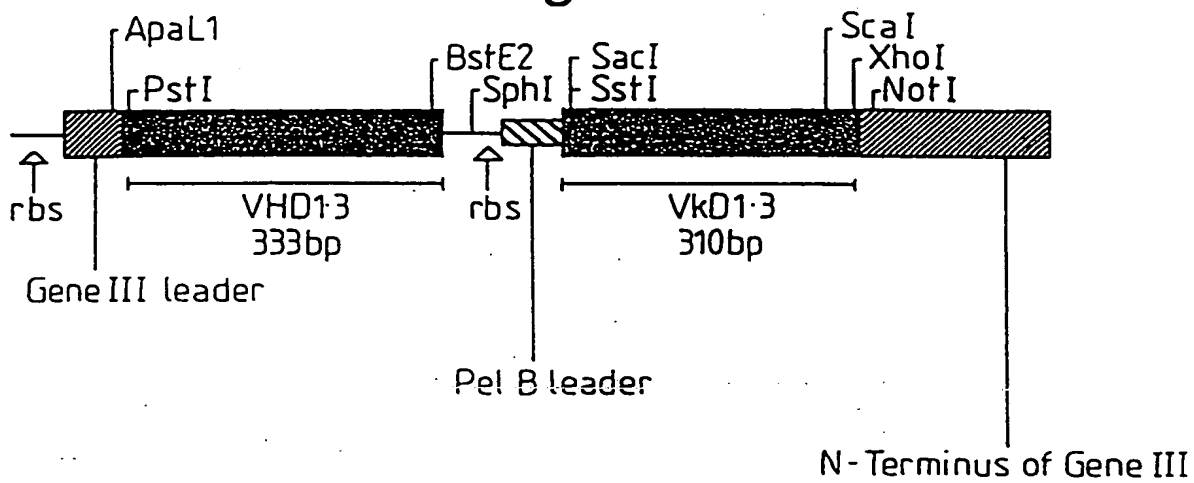


Fig.46.

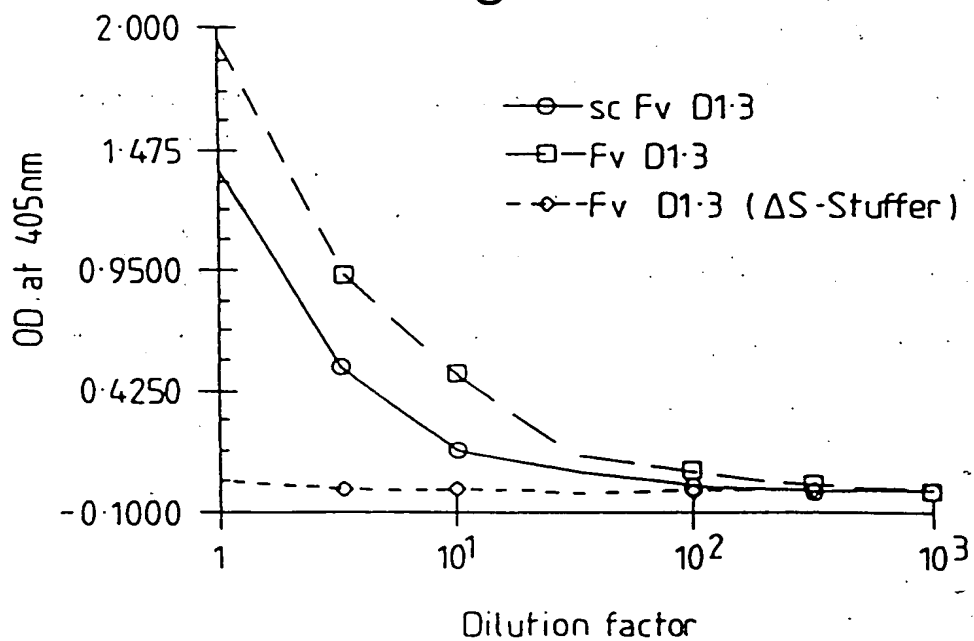




Fig.47.

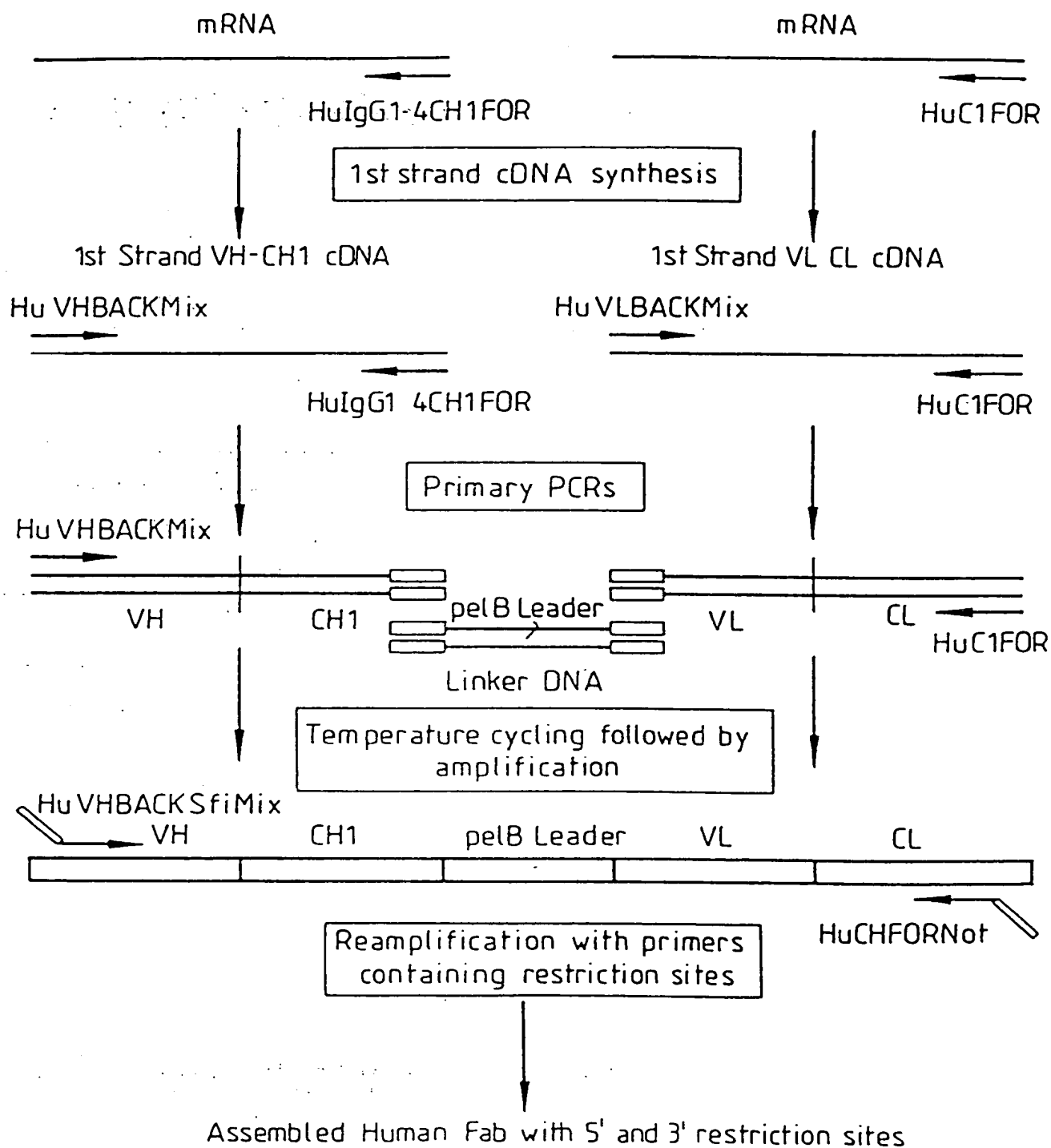


Fig.48(i)

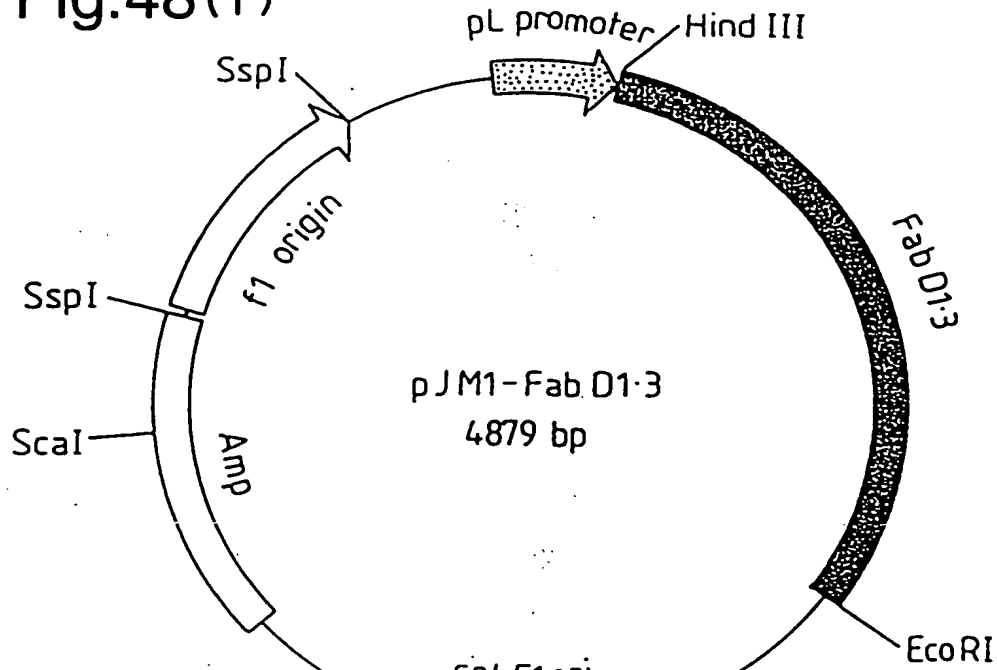


Fig.48(ii)

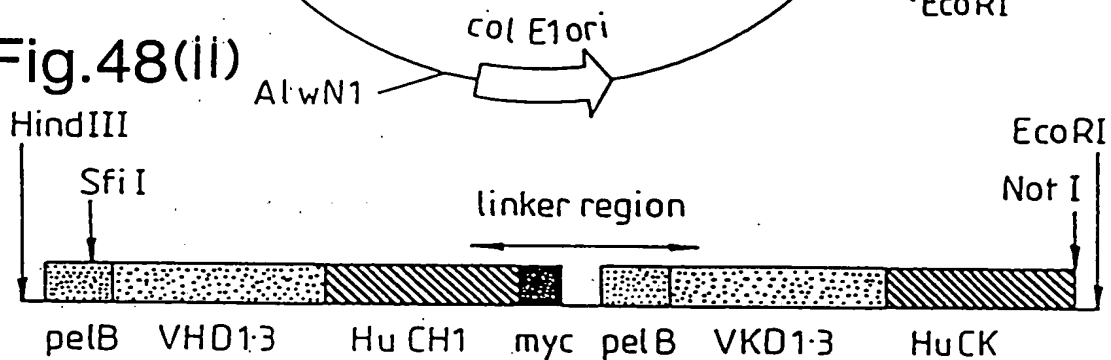
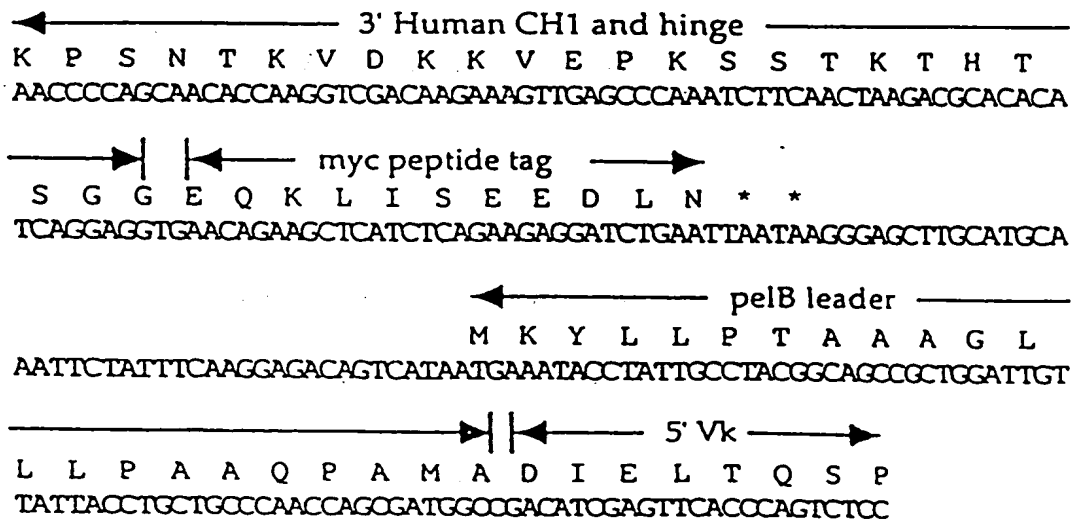
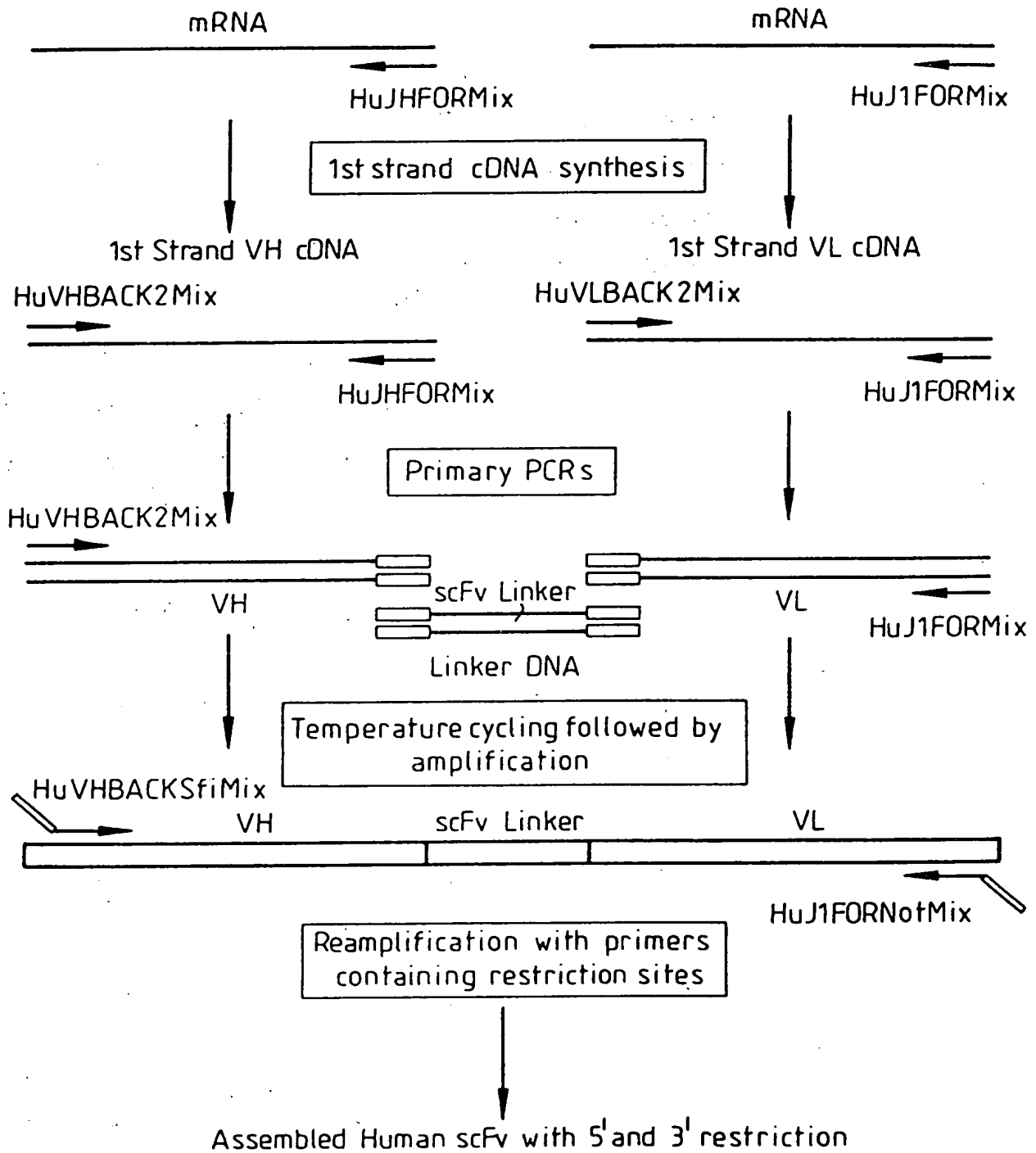


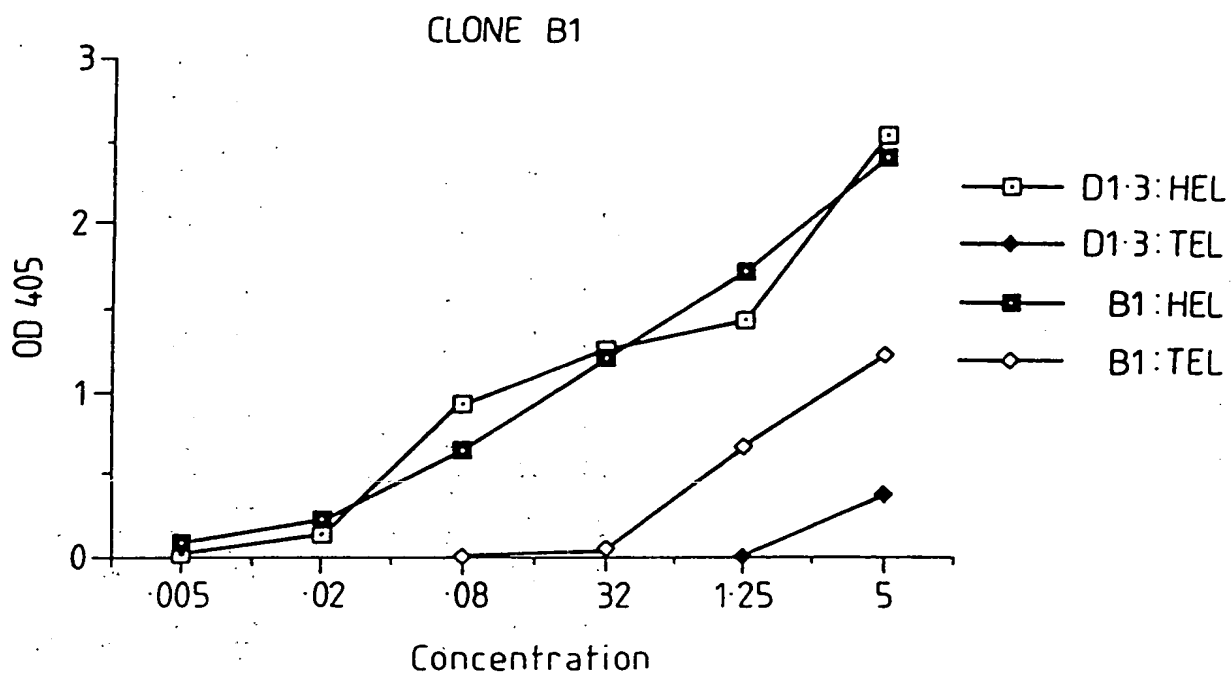
Fig.48(iii)



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# Fig.50(i)



# Fig.50(ii)

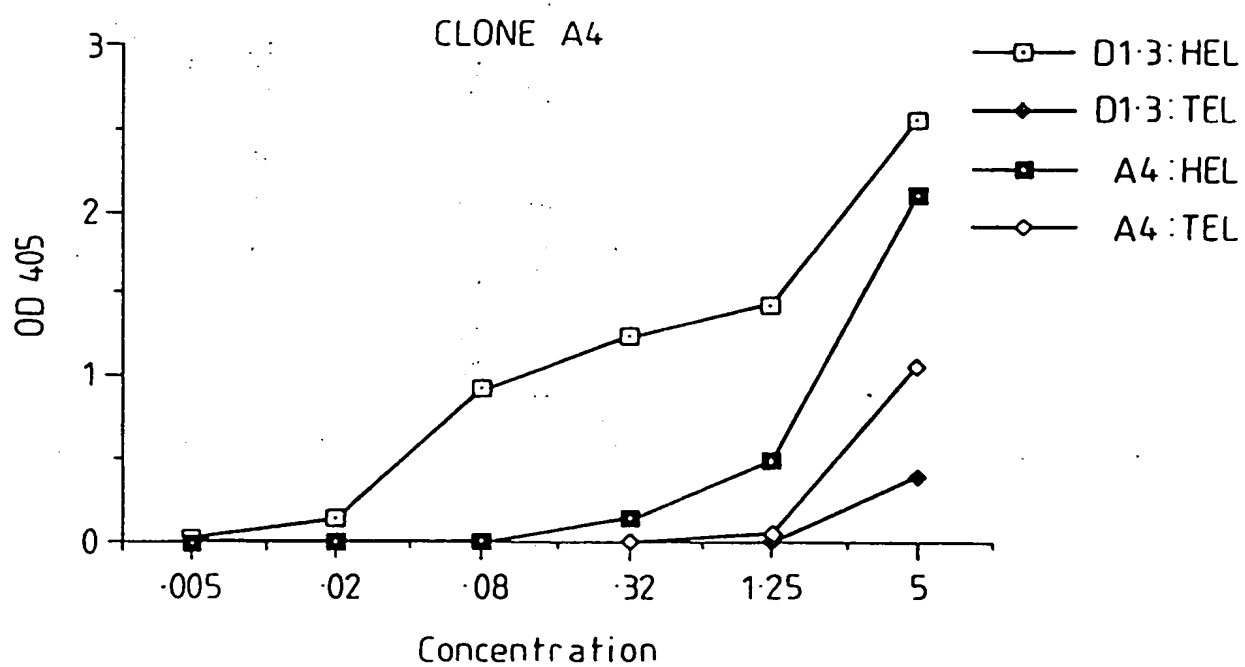


Fig.52.

CDR 1

CDR 2

D1.3 DIQMTQSPASLSASVGETVTITCRASGNIHNYLA WYQQKQKSPQLLVYTTTLAD  
M1F DIELTQSPSSLASLGERVSLTCRASQDIGSSLN WLQQEPDGTIKRLIYATSSLDS  
M21 DIELTQSPALMAASPGEKVTITCSVSSSISSNLHWYQQKSETSPKPWIYGTSNLAS

CDR 3

D1.3 GVPSRFGSGSGTQYSLKINSLQPEDFGSYQCQHFWSPTPTFGGGTKLEIKR  
M1F GVPKRFSGSRGSDYSLTISSESEDFVDYVCLQYASSPWTFFGGGTKLELKR  
M21 GVPVRFSGSGTSYSLTISSEAEADAATYCCQWSSYPPLTFGAGTKLEIKR

Fig.51.

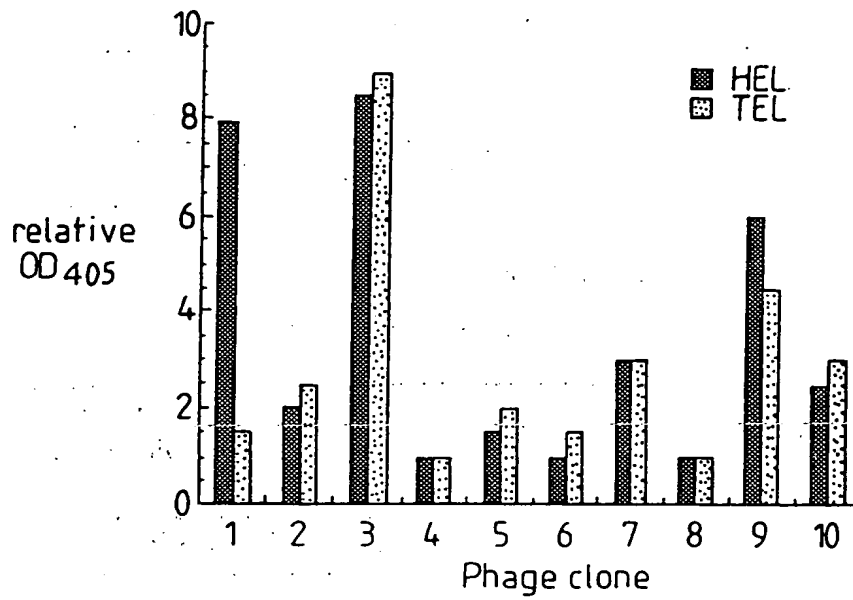


Fig.53.

